Remarks

Claims 2-4, 10-17, 31 and 33-36 are pending in the application upon entry of the above amendment. Claims 34 and 35 have been withdrawn from consideration. Claim 36 is new.

Claims 2, 3 and 33 have been amended to indicate that the starch utilized in the claimed methods is a *semi-crystalline* starch. Support for the amendment is found in the specification at page 21, lined lines 10-11, for example. Support for new claim 36 is found in the specification at page 16, lines 22-25. Withdrawn claims 34 and 35 have been amended to recite that the administered food product is a semi-crystalline starch which is a heat moisture-treated starch or annealing-treated starch, support for which is found in the specification at page 21, lines 10-13, and page 21, line 24 to page 23, line 26.

Claim 31 has been amended to independent form, and recites the composition of the therapeutic food composition contained in the claimed therapeutic food kit.

Reconsideration and allowance is requested in view of the above changes and the and the following remarks.

Acknowledgement of Telephonic Interview

Applicants thanks Examiners Nannette Holman and Brandon Fetterolf for the courtesy extended to the undersigned attorney during the telephonic interview of September 10, 2009.

No exhibit was shown. Claims 2 and 3 were discussed in particular, and all independent claims were discussed generally. A claim amendment was proposed substantially in the form set forth above.

The undersigned attorney presented reasons why the claimed invention was not anticipated or obvious in view of Bohrmann *et al.* The undersigned pointed out that the ingestion of the food product described by Bohrmann *et al.* would not inherently result in the claimed invention, for the reasons discussed in later in this paper. The undersigned further explained how Bohrmann does not provide for ingestion of a food product which contains a *semi-crystalline* heat moisture-treated starch or annealing-treated starch, for the reasons discussed in later in this paper. The undersigned indicated that the various secondary references asserted against the dependent claims did not cure the deficiencies of the primary reference. The

undersigned indicated that rejoinder of claims 34 and 35 would be requested in view of the amendments thereto, and that claim 36, newly presented, is properly grouped with the claims presently subject to examination. No agreement as to allowance was reached.

Response to 35 U.S.C. 102 Rejection

Claims 1-3, 5, 17 and 33 have been rejected as allegedly anticipated by Bohrmann *et al.* ("Bohrmann"). Claims 1 and 5 were previously cancelled. Claim 2 is directed to a method of treating hypoglycaemia in an individual in need of such treatment, comprising administering a therapeutic food composition comprising a semi-crystalline starch, which starch is a moisture-treated starch or an annealing-treated starch. As indicated previously, Bohrmann is simply concerned with providing methods of preparing thickened food products, in particular dry food products which ultimately are added to boiling aqueous liquid and cooked therein. Bohrmann provides no teaching whatsoever of the use of such food products in the treatment of any disease, let alone hypoglycaemia. The examiner indeed agrees that Bohrmann does not mention the claimed use.

Notwithstanding the absence of any teaching of treating hypoglycaemia in Bohrmann, or any other disease, the rejection alleges that Bohrmann inherently anticipates the claimed invention. The examiner alleges that the ingestion of the Bohrmann product would inherently lead to an increase in glucose levels, which would inherently result in treatment of hypoglycemia.

Anticipation by inherent disclosure is generally only appropriate when the reference discloses prior art that must *necessarily* include the unstated limitation. Even assuming *arguendo* that the food product of Bohrmann could be effective in treating hypoglycemia, which is not admitted, not all people who would ingest the food product of Bohrmann would suffer from hypoglycemia. The mere fact that a certain thing may result from a given set of circumstances, *i.e.*, that some of the population who may ingest the composition of Bohrmann may suffer from hypoglycemia, is insufficient ground on which to premise a rejection for inherent anticipation.

To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." *Continental Can Co. v. Monsanto Co.*, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991). Thus, "anticipation by inherent disclosure is appropriate only when the reference discloses prior art that must *necessarily* include the unstated limitation, [or the reference] cannot inherently anticipate the claims." *Transclean Corp. v. Bridgewood Servs., Inc.*, 62 USPQ2d 1865, 1871 (Fed. Cir. 2002) (emphasis in original).

It is not sufficient if a material element or limitation is "merely probably or possibly present" in the prior art. *Trintec Indus., Inc. v. Top-U.S.A. Corp.*, 63 USPQ2d 1597, 1601 (Fed. Cir. 2002). As the CCPA stated in *In re Oelrich*, 212 USPQ 323, 326 (CCPA 1981) (quoting *Hansgirg v. Kemmer*, 40 USPQ 665, 667 (CCPA 1939)) (internal citations omitted):

> Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.

See also, Mehl/Biophile International Corp. v. Milgraum, 52 USPQ2d 1303, 1305-06 (Fed. Cir. 1999); *In re Robertson*, 49 USPQ2d 1949, 1951 (Fed. Cir. 1999).

"In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the alleged inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd.Pat.App. & Int.1990). Examiner has not, and cannot, make such a showing.

The rejection does not establish that the alleged inherent characteristic – treatment of hypoglycaemia – necessarily flows from the teachings of Bohrmann. There is no suggestion in Bohrmann to administer the food product described therein to individuals who are either suffering from hypoglycaemia (claim 2) or who are susceptible to hypoglycaemia (claim 3). A mere possibility of such result, or even a probability of that result, is insufficient to give rise to anticipation. *Trintec Indus., supra.* Only a result that *inevitably* flows from the teaching of a reference may inherently anticipate. Even assuming *arguendo* that ingestion of the food product of Bohrmann could be effective in treating hypoglycemia, which is not admitted, the treatment of

hypoglycaemia, or the prevention thereof in susceptible individuals, would not inevitably flow from a person's ingestion of that product, since that individual may or may not suffer from hypoglycaemia, or may or may not be susceptible to hypoglycaemia. Bohrmann does not inherently anticipate the invention of claims 2, 3 and 17.

Claims 2, 3 and 17 are further distinguished in view of the amendment to claims 2 and 3. Those claims have been amended such that the therapeutic food composition comprises a semi-crystalline starch which is a heat moisture-treated starch or annealing-treated starch. Thus, the composition as described in these claims must not only comprise a starch which is a heat moisture-treated starch or annealing-treated starch, but must be a starch which is *semi-crystalline* in nature.

When the food composition of Bohrmann is ingested, the result would not be ingestion of a semi-crystalline starch. Bohrmann relates to a dry food product containing a thickening agent, which food product is prepared for ultimate consumption by adding it directly to boiling aqueous liquid and cooking it. The thickening agent may consist of a heat-moisture-treated potato starch. The heat moisture-treated starch can control the gelatinization process of the food product and make thickened products, but in the process the starch becomes gelatinized when the product is cooked. Thus, to the extent the Bohrmann food product may contain a heat-moisture-treated starch, that starch is gelatinized by cooking before ingestion. The person ingesting the food product would not be ingesting a starch which is semi-crystalline in nature, since the semi-crystalline nature of the starch granules is lost by gelatinization when the food product is cooked in boiling aqueous liquid. Hence, claims 2, 3 and 17 are not anticipated by the ingestion of the foodstuffs of Bohrmann because the foodstuffs do not contain semi-crystalline starch when ingested.

Claims 2, 3 and 17 are not anticipated by the ingestion of the foodstuffs of Bohrmann for yet another reason. The ingested foodstuff of Bohrmann, comprising a gelatinized starch, would not be capable of effectively functioning to treat hypoglycemia, or treat an individual susceptible to hypoglycemic episodes. To control hypoglycemia (beyond that of the normal digestion process, i.e. 90-120 minutes for gelatinized starch), the semi-crystalline nature of the starch granules must be retained. If heat moisture-treated or annealing-treated starches are consumed

for hypoglycemia management, they must not be exposed to temperatures above 45°C to 55°C in an excess of water; otherwise they cannot exert any hypoglycemic control. Above this temperature in an excess of water, gelatinization is initiated, and the semi-crystalline structure necessary for hypoglycemia control is lost. The loss of the semi-crystalline structure upon boiling in aqueous liquid is clearly apparent in Bohrmann.

There is no teaching or suggestion in Bohrmann of ingesting the described food product without first boiling it in aqueous liquid. Thus, there is no disclosure by Bohrmann of the heat moisture-treated starch utilized as a thickening agent to ever be ingested in its ungellatinized semi-crystalline form. The boiled product would not inherently contain semi-crystalline starch material and would not be capable of performing the function of the claimed invention in controlling hypoglycemia. This is apparent from the following discussion.

Starch is synthesized in plants in discrete granules, which are formed in the plants' amyloplasts. The granules are roughly spherical and range from about 5 μm in diameter to greater than 50 μm in diameter; the granules contain molecules of amylopectin and amylose (specification, page 18, lines 19-21).

Native starch granules are relatively indigestible in the human small intestine because the semi-crystalline granules (where the crystallinity is imparted by the laminates of amylopectin and possibly some amylose double helices) are arranged in ordered arrays (specification, page 18, line 19- page 20, lines 8). This is a very concentrated form of energy for the plants. For starch granules to be fully, easily and quickly digestible by humans however, they must be cooked in water, which disrupts the crystalline regions and gelatinizes the starch (specification, page 19, lines 29-32). The cooking process generally involves vigorously heating the starch in an excess of water (*e.g.* greater than 100 ml of water per gram of starch) to facilitate the rupture of internal double bonds Tester, R. F. and Karkalas, J. (1997) Quality and functionality of starches, pp 123-128, *in: Proceedings of the 47th Australian Cereal Chemistry Conference, Perth, Australia.* ISBN 0909589941) (**Appendix A**). Excess water acts as both a plasticizer and a site of hydrogen bonding with the gradually unraveling of double helices in the starch . As this happens, the granules swell and the viscosity of the mixture increases, thereby providing a thickening effect (Tester, R. F. and Morrison, W. R. (1990). Swelling and gelatinisation of cereal

starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chemistry 67 (6), 551-557*) (**Appendix B**). At this point, which is initiated around 45°C for most starches in excess water, the amorphous starch becomes fully, easily and quickly digestible (Holm, J., Lundqvist, I., Björck, I., Eliasson, A. C. and Asp, N. G. (1988) Degree of starch gelatinization, digestion rate of starch in vitro and maetabolic response in rats. *American Journal of Clinical Nutrition 47, 1010-1016*) (**Appendix C**). The starch is gelatinized.

Typically, digestion time for gelatinized starches is around 90-120 (less than 180) minutes (Holm, J. and Björck, I. (1992) Bioavailability of starch in various wheat-based bread products: evaluation of metabolic responses in healthy subjects and rate and extent of in vitro starch digestion. *American Journal of Clinical Nutrition 55, 420-429*) (**Appendix D**). This is far too rapid and abrupt a digestion time and profile to provide for effective control of hypoglycemia. Gelatinized starches are not effective in controlling hypoglycemia.

Natives starches are similarly ill-suited for controlling hypoglycemia . Native starches can be eaten by man even though only a limited amount of digestion (by alpha-amylase) occurs. That digestion which can occur does so rapidly (after approximately 90-120 minutes) and causes a peak in blood glucose followed by a rapid insulin response which attempts to balance the elevated blood glucose, for example, by converting it to glycogen for storage in the liver (Ross, S. W., Brand, J. C., Thorburn, A. W. and Truswell, A. S. (1987) Glycaemic index of processed wheat products. *American Journal of Clinical Nutrition 46, 631-635*) (**Appendix E**). For native waxy maize starches, it has been shown recently that any residual matter is digested slowly over about 5 hours where some more glucose is liberated into the blood as a consequence of digestion (Sands, A., Leidy, H. J., Hamaker, B. R., Maquire, P. and Campbell, W. W. (2009) Consumption of the slow-digesting waxy maize starch leads to blunted plasma glucose and insulin response but does not influence energy expenditure or appetite in humans. *Nutrition Research 29, 383-390*) (**Appendix F**). While the aforementioned authors indicate that waxy maize starches could be used to treat hypoglycaemia, on its own the native starch has limited value to treat extended hypoglycemia due to the initial 90 minute glucose spike, the short digestion time (up to 4 hours) and the metabolic consequences, for example, the associated insulin response. Hence, there is a need to delay the duration of digestion, and to reduce the initial glucose spike.

The starches administered according to the present invention have been either heat moisture-treated or annealing-treated, but not gelatinized as in the starches in Bohrmann. Hence, they retain a semi-crystalline structure. Heat moisture-treatment and annealing-treatment have specific meanings in carbohydrate chemistry and these meanings are explicitly disclosed in the specification at page 22, line 6 to page 23, line 23. Those treatments do not result in gelatinization. Heat moisture treated starch is typically produced by exposing moist starch (e.g. 15-30% moisture) to temperatures of e.g. 95°C to 130° for periods up to 30 hours, typically 16-24 hours. For example, heat moisture treated starch for use in the invention may be produced by thermally treating starch in a sealed container under the following conditions: 20% moisture and 105°C for 16 hours. The treated starch may then be cooled to room temperature, air-dried and then passed through 300μm sieve. See the specification, page 22, lines 6-16.

Annealing is a process in which starch granules are treated for a relatively long time in excess amounts of water at a temperature slightly higher than room temperature. Typically, annealing of starch involves incubation of starch granules in water (>40% w/w), for a time period in the range 1 hour to 10 days at a temperature between the glass transition and the gelatinization temperature. Preferred annealing conditions are less than 10°C below the onset of gelatinization temperature, in excess water for up to 7 days. See the specification, page 23, lines 13-23.

Applicants have discovered that semi-crystalline heat moisture-treated or annealing-treated starches have a lower initial glucose spike (lower after 90 minutes), and longer digestion duration than either gelatinized starch or native starch. The lower initial glucose spike and longer digestion duration (up to 10 hours) make semi-crystalline heat moisture-treated or annealing-treated starches useful for treating hypoglycemia or preventing or decreasing hypoglycemic episodes in individuals susceptible to such episodes. Applicants (and subsequently others) have discovered that that semi-crystalline heat moisture-treated or annealing-treated starches also have a better lactate response, and a positive, i.e., less extreme, insulin response (Bhattacharya, K., Orton, R. C., Qi, X., Mundy, H., Morley, D. W., Champion, M. P., Eaton, S., Tester, R. F. and Lee, P. J. (2007) A novel starch for the treatment of glycogen storage disease. *Journal of Inherited Metabolic Disease 30, 350-357*) (**Appendix G**). Hence, the

semi-crystalline heat moisture-treated or annealing-treated starches are useful for treating hypoglycemia or preventing or decreasing hypoglycemic episodes in individuals susceptible to such episodes.

Claims 2, 3 and 17 are not anticipated by the ingestion of the foodstuffs of Bohrmann because the foodstuffs neither contain semi-crystalline starch when ingested; nor would they be capable of functioning to control hypoglycemia. The unique properties of semi-crystalline heat moisture-treated or annealing-treated starch provides the starch with the ability to be slowly digested, and therefore provides glucose to a sufferer of hypoglycemia, over a period of 10 hours, whereby serum glucose levels are maintained compared to starches which are not ungellatinized heat moisture-treated or annealing-treated starches.

Claim 33 is also not anticipated by Bohrmann. Claim 33, as amended, is directed to a sports nutrition foodstuff comprising a semi-crystalline starch wherein the starch is a waxy starch, a hydrothermally treated starch, or a combination thereof. For the reasons stated above, Bohrmann does not teach the direct utilization of the starch-containing thickening agent disclosed therein for direct ingestion. To the extent that anything is taught for ingestion, it is a foodstuff that has been boiled, which would destroy the semi-crystallinity of any starch contained therein. Bohrmann does not anticipate claim 33.

Accordingly, claims 2, 3, 17 and 33 are not anticipated by Bohrmann. Withdrawal of the Section 102 rejection is respectfully requested.

Response to 35 U.S.C. 103 Rejections

Rejection of claims 4, 13-14 and 31 over Bohrmann in view of Schmeidel *et al* ("Schmeidel")

Claims 4 and 13-14 have been rejected as being unpatentable over Bohrmann in view of Schmiedel. While not stated in the first sentence of the rejection (where the claims under rejection are identified), it is understood that the rejection extends to claim 31, as claim 31 is referenced in the body of the rejection. Thus, applicants understand the Section 103 rejection to be directed to claims 4, 13, 14 and 31.

The Examiner alleges that Bohrmann is directed to a food product and discloses the use

of heat moisture treated starches and the use of a food composition that contains greater than 50 grams of starch. The Examiner goes onto suggest that the food product of Bohrmann is understood to meet the limitation of "kit" in claim 31, and that is it known that the consumption of starch by humans increases glucose levels which the Examiner alleges would treat hypoglycemia. The Examiner goes on to explain that Bohrmann differs from the prior art insofar as it does not disclose a waxy starch, a feature of claims 4 and 14.

The Examiner alleges that Schmiedel discloses a food composition with a waxy maize (corn) starch and a hydrothermally treated starch. Furthermore, the Examiner believes that the waxy starch has an amylose content of less than 10%, the significance being that the present specification defines a "waxy" starch as containing <20% amylose (80% amylopectin), and that Schmiedel discloses the process of using hydrothermally treated waxy starches to improve the quality and quantity of products made. Examiner acknowledges that Schmiedel differs from the claimed invention in that it does not disclose heat moisture-treated or annealing-treated starches. Nevertheless, it is alleged that it would have been obvious to have used the waxy starch of Schmiedel in the food product of Bohrmann, motivated by the desire to produce a product of improved quality at a large quantity as allegedly disclosed by Schmiedel.

The deficiencies of Bohrmann are discussed above with respect to claim 3, from which claims 4, 13 and 14 depend. Bohrmann does not disclose a method of treating an individual susceptible to hypoglycemic episodes to prevent or decrease those episodes by administering a food composition comprising a semi-crystalline starch which is a heat moisture-treated starch or annealing-treated starch. This is because Bohrmann does not disclose ingestion of a semi-crystalline heat moisture-treated starch or annealing-treated starch. The boiled product of Bohrmann, in which the starch is gelatinized. would not inherently contain semi-crystalline starch material and would not be capable of performing the function of the claimed invention.

Schmiedel does not cure the deficiencies of Bohrmann. It appears that the Examiner admits that the waxy starch of Schmiedel is not a heat moisture-treated starch or annealing-treated starch, irrespective of crystallinity. Thus, substitution of the waxy starch for the heat moisture-treated starch of Bohrmann would lead *away from* the claimed invention, since the resultant would not be a composition containing a heat moisture-treated starch or annealing-

treated starch.

In any event, Schmiedel teaches the preparation of (a retrograded form of) resistant starch (*i.e.*, a starch derivative which is resistant to digestion/metabolism in the small intestine) to be fermented in the colon and hence act as a prebiotic. For the processes described in Schmiedel, the starches must be gelatinized before treating with enzymes in order to debranch the starches (Schmiedel, par. 002-0026) and thus allow retrogradation of the α-glucans, isolated initially from starch granules. Therefore, even assuming the combination of Bohrmann and Schmiedel is proper, which is not admitted, the resultant is clearly not the claimed invention since the resultant is not a semi-crystalline starch, but rather a gelatinized starch, which is then modified further.

The resistant starch of Schmiedel is not directed towards or intended to act as a hypoglycemia therapy as the material would not be digested in the small intestine but would travel to the colon. In the colon, the material of Schmiedel produces short-chain fatty acids (SCFAs) for colonic health. *See*, Schmiedel, par. 002-0026; see also see the discussion of resistant starches in the present specification at page 20, lines 3-8. In order to maximize the yields obtained after debranching by enzymes, Schmiedel uses waxy starches for this purpose. The crystallinity of the waxy starch is lost by gelatinization by heating and treatment with enzymes (which leads to retrogradation of the isolated α-glucans). In contrast, the starches of the present invention are utilized in a semi-crystalline state, not a gelatinized state. The heat moisture treatment or annealing treatment described in the present invention is employed to enhance the crystallinity of the native granules, but not to control gelatinization or subsequent dispersion, or thickening or accessibility to enzymes for further modification during processing. Since the process of retrogradation described by Schmiedel follows gelatinization, the Schmiedel process is opposite to that taught by the present invention, and results in a loss of starch granule semi-crystallinity.

One of ordinary skill in the art would not have been motivated to substitute a waxy starch in the food product of Bohrmann in order to provide a starch which can be used to treat hypoglycemia. There is no teaching or suggestion in either document of a semi-crystalline heat moisture treated or annealing treated starch for the treatment of hypoglycemia. Furthermore, neither document teaches or suggests that such a composition would extend the duration of time

for which starch will release glucose into the blood stream.

Thus, the subject matter of claims 4 and 13-14 would not have been obvious over Bohrmann in view of Schmiedel.

Claim 31 had been amended to recite the features of the therapeutic food composition contained in the therapeutic food kit. Claim 31 therefore recites a therapeutic food composition comprising a semi-crystalline starch which is a heat moisture-treated starch or annealing-treated starch. For the reasons stated above, neither Bohrmann nor Schmiedel, alone or in combination, describe such a food product.

The rejection of claim 31 alleges that patentable weight need not be given to the non-functional descriptive matter in the claims, i.e., the printed instructions, absent a new and nonobvious functional relationship between the printed matter and the substrate, citing MPEP2106.01.

Here, there is a new and unobvious functional relationship between the printed matter and the substrate. The printed matter provides instructions for treating hypoglycaemia or preventing or decreasing hypoglycemic episode(s) by ingesting the therapeutic food composition. For the reasons discussed above in connection with the anticipation rejection over Bohrmann and the obviousness rejection over Bohrmann or Schmiedel, the use of a semi-crystalline heat moisture-treated starch or annealing-treated starch for the treatment of hypoglycemia is both novel and nonobvious. Hence, printed instructions directed to that use provide a new and nonobvious functional relationship between the printed matter and the composition contained in the kit.

The printed instructions must be given patentable weight, and therefore serve to distinguish the kit over the asserted prior art. Reconsideration and withdrawal of the rejection of claim 31 is respectfully requested.

Rejection of claims 10-11 and 15-16 over Bohrmann in view of Kaufman

Claims 10-11 and 15-16 have been rejected as being unpatentable over Bohrmann in view of Kaufman.

The Examiner alleges that Kaufman discloses a therapeutic food composition containing starch for treatment of diabetic patients to prevent hypoglycemic episodes and diminish

fluctuations in blood sugar levels. The Examiner also alleges that Kaufman further discloses treating patients having glycogen storage disease and Type I or Type II diabetes. Also, the Examiner believes that Kaufman discloses maintaining blood sugar levels above 16 milligrams per deciliter for as long as 8 to 9 hours, which allegedly meets the limitations of claims 15 and 16.

The Examiner acknowledges that the invention of the present application differs from Kaufman insofar as it does not disclose a therapeutic food composition containing heat moisture-treated or annealing-treated starch. However, the Examiner believes that it would have been obvious to one of ordinary skill in the art at the time of the invention to use the heat moisture treated starch of Bohrmann as the food product of Kaufman, motivated by desire to diminish fluctuations in blood sugar levels and to treat glycogen storage disease and diabetes as disclosed by Kaufman.

The deficiencies of Bohrmann are discussed above with respect to claim 3. Claims 10-11 and 15-16 depend directly or indirectly from claim 3. As indicated above, Borhmann does not disclose a method of treating an individual susceptible to hypoglycemic episodes to prevent or decrease those episodes by administering a food composition comprising a semi-crystalline starch which is a heat moisture-treated starch or annealing-treated starch. Bohrmann does not disclose ingestion of a semi-crystalline heat moisture-treated starch or annealing-treated starch. The boiled product of Bohrmann, in which the starch is gelatinized, would not inherently contain semi-crystalline starch material and would not be capable of performing the function of the claimed invention. Bohrmann merely describes a heat moisture-treated starch as a thickener for a food product which is boiled.

Kaufman does not cure the deficiencies of Bohrmann. Kaufman teaches the preparation of formulations comprising native maize starches. The native maize starches of Kaufmann are not semi-crystalline starches that are heat moisture-treated or annealing-treated, and this fact is recognized in the office action. There is no suggestion in either Bohrmann or Kaufman that semi-crystalline starches that are heat moisture-treated or annealing-treated have any utility in the treatment of hypoglycemia, glycogen storage disease, or Type I or Type II diabetes, or that they would be able to maintain blood glucose levels as disclosed in claims 15 and 16.

The rejection appears to suggest that there would have been incentive to substitute the moisture-treated starch of Bohrmann for the maize starch in the disclosure of Kaufman. The disadvantage of conventional starch, such as maize starch, in the treatment of conditions such as glycogen storage disease, are discussed in the present specification at page 10. The inventors have developed the present application because medical practitioners requested that they do so, as they noted that native maize starches (as described in Kaufman) were used with very limited effect for hypoglycemic therapy, since they could only provide fewer hours glucose release during digestion, and since they provided a large initial (90 minute) glucose spike which produced an unfavorable insulin and lactate response (Bhattacharya, et al. (**Appendix G**); Sands, et al. (**Appendix F**)).

The inventors discovered that heat moisture-treated or annealing-treated starches extended the digestion/glucose release time of native starches (up to 10 hours glucose release to maintain blood sugar levels sufficient to prevent hypoglycemia), had a smaller 90 minute glucose response, and a much more favorable metabolic (i.e. insulin and lactate) response.

There is nothing in the disclosure of Kaufman that teaches or suggests heat moisture treatment or annealing treatment of semi-crystalline starches, let alone that such treated starches would be advantageous over normal maize starches. There is nothing in the disclosure of Kaufman to lead the skilled artisan to make the substitution asserted in the rejection. There is nothing in the disclosure of Kaufman or Bohrmann to suggest that semi-crystalline starches that are heat moisture-treated or annealing-treated should or could replace the maize starch in Kaufman, or would have advantageous properties over maize starch in the treatment of hypoglycemia. It is only with hindsight and the benefit of applicants' disclosure that such a substitution would be envisioned, for the advantages described in the specification.

There is no suggestion in either Bohrmann or Kaufman that semi-crystalline starches that are heat moisture-treated or annealing-treated have any utility in the treatment of hypoglycemia, glycogen storage disease, or Type I or Type II diabetes, or that they would be able to maintain blood glucose levels as disclosed in claims 15 and 16.

There is no motivation apparent from Bohrmann or Kaufman to attempt to treat any form of hypoglycemia using semi-crystalline heat moisture-treated or annealing-treated starches.

Furthermore, there is no suggestion in the asserted references that such starches would have an extended digestion time such that, for example, would allow them to be used over night such that a patient with hypoglycemia does not have to be fed glucose naso-gastrically, intravenously or be woken.

In addition, there is no suggestion or motivation in Kaufman to replace the native starches disclosed therein with any form of heat moisture-treatment or annealing-treatment in order to obtain the product of the present invention which has a much longer digestion time and a much more favorable metabolic response thereby making the product of the present invention much more suitable for the treatment of diseases such as glycogen storage disease, Type I and Type II diabetes and the like.

Thus, the subject matter of claims 10-11 and 15-16 would not have been obvious over Bohrmann in view of Kaufman.

Rejection of claim 12 over Bohrmann in view of Hansson *et al.* ("Hansson")

Claim 12 has been rejected as being unpatentable over Bohrmann in view of Hansson. The Examiner alleges that Hansson is directed towards a composition of heat treated starch for the prevention of hypoglycemia in patients with diabetes or liver disease, and discloses a method for stabilizing the blood sugar levels and avoiding the oscillation between an unhealthy high and low blood sugar levels. The Examiner acknowledges that Hansson differs from the present invention insofar as it does not disclose a heat moisture-treated or annealing-treated starch. However, the Examiner alleges that it would have been obvious to one of ordinary skill in the art at the time of the invention to use the heat moisture-treated starch of Bohrmann as the food product of Hansson motivated by the desire to stabilize the blood sugar level and avoid the oscillation between unhealthy high and low blood sugar levels in patients with liver disease as disclosed by Hansson.

Hansson teaches granulate particles that contain starch for treatment of hypoglycemia. Bohrmann does not teach the use of semi-crystalline heat moisture treated or annealing treated starches in the treatment, maintenance or stabilization of blood sugar levels in the treatment of hypoglycemia or liver disease. There is no motivation in Bohrmann to use starches in the heat

moisture treated or annealing-treated state for any purpose, as Bohrmann specifically teaches that the starches should be gelatinized (i.e. used as thickening agents) before they are ingested. There would accordingly be no motivation for the skilled artisan to substitute the heat-moisture treated starch of Bohrmann for the granulated particulate starch of Hansson. The heat-moisture treated starch of Bohrmann is utilized merely as a thickening agent in that disclosure. There would have been no motivation to utilize a mere thickener to replace a granulated starch which is used in Hansson to stabilize the blood sugar level and avoid the oscillation between unhealthy high and low blood sugar levels in patients with liver disease as disclosed by Hansson.

The combination of Hansson and Bohrmann would not lead the skilled artisan to the present invention. There would be no motivation to combine the teachings of Hansson and Bohrmann. Even if one were to combine the teachings of Hansson and Bohrmann, they would not arrive at the present invention.

Thus, the subject matter of claim 12 would not have been obvious over Bohrmann in view of Hanson.

Request for Rejoinder of Claim 34 (Group II) and Claim 35 (Group III)

Claims 34 and 35, directed to a method of treating glycogen storage disease and liver disease, respectively, were added in the Amendment filed January 26, 2009. Those claims have been withdrawn from consideration by the current office action, because an action has been received on the claims of what is now considered Group I (claims 2-4, 10-17, 31 and 33). Restriction has been required to the claims of Group I because the technical feature linking all claims is the administering a food composition. It is further alleged that the feature does not comprise a *special* technical feature, because it is disclosed in Bohrmann.

Claims 34 and 35 have been amended herein to recite that the therapeutic food composition administered is a semi-crystalline starch which is a heat moisture-treated starch or annealing-treated starch, which is the same feature now set forth in the other independent method claims in the application, claims 2 and 3. For the reasons discussed above in connection with the rejection of claims 2 and 3 over Bohrmann, the latter document neither anticipates nor renders the administration of a semi-crystalline starch which is a heat moisture-treated starch or

annealing-treated starch, for the treatment of any disease. As claims 34 and 35 share this technical feature with claims 2 and 3, and because the feature distinguishes over the prior art, claims 34 and 35 are properly joined in the application.

Claim 36 is Properly Grouped with the Claims Presently Examined

New claim 36 is directed to a treating method of treating Type I diabetes or Type II diabetes by administering a therapeutic food composition comprising a semi-crystalline starch which is a heat-moisture treated starch or annealing-treated starch. For the same reasons set forth above regarding the rejoinder of claims 34 and 35, claim 36 is properly joined in the application.

Conclusion

The claims remaining in the application are believed to be in condition for allowance. An early action toward that end is earnestly solicited.

Respectfully submitted,

XIN QI *et al*

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APPENDIX A

CONFERENCE PROCEEDINGS

CEREALS '97

47TH CEREAL CHEMISTRY CONFERENCE

Quality grains –
Meeting the needs of consumers

14 - 19 September 1997

Novotel Langley Hotel
Perth, Western Australia

Cereals '97

Proceedings of the
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QUALITY AND FUNCTIONALITY OF STARCHES

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Starch is synthesised by plants as an energy storage reserve. It is formed in cellular organelles, called amyloplasts, as roughly spherical granules. The size and shape of these granules are indicative of the botanical origin (Table 1).

Table 1 Size and shape of some common starch granules

Botanical origin	Distribution and Shape	Diameter (μm)
Barley, Rye & Wheat	Bimodal/Round/Oval	2-35
Maize, Millet & Sorghum	Uni-/Bimodal/Polyhedral	4-25
Oats and Rice	Uni-/Bimodal/ Compound/Round	3-8
Horse Bean	Unimodal/Round/Oval	10-35
Smooth Pea	Unimodal/Round	4-15
Manioc	Unimodal/Oval	5-35
Potato	Unimodal/Oval	10-100

Most starches have a unimodal distribution of granule size, although the *Triticeae* have a definite bimodal distribution of small B-type (*circa* 1-10μm) and large A-type granules (*circa* 11-35μm diameter). There is often evidence of this bimodal distribution in other cereal starches although not as obvious as in the *Triticeae*. Certain starches (like oats and rice) may contain compound granules consisting of clusters of small granules.

In highly purified starches (where surface contaminants like lipids and proteins from non-starch tissues have been removed) there is about 84-90% α-glucan, 10-15% moisture, up to 1.5% lipid (in cereal starches only) and less than 0.5% protein. The α-glucan comprises amylose and amylopectin only. We have extensively researched the so-called 'intermediate material' fraction reportedly present in oat starches but have never found any evidence of its existence (Tester and Karkalas, 1996). The structure of these α-glucans is under genetic control and depends on the botanical origin. The moisture content of air equilibrated cereal starches is about 11-12%, and about 14-15% in tuber starches. There is a strong positive correlation between amylose and lipid content in cereal starches. The lipid tends to be exclusively lysophospholipid in starches extracted from the *Triticeae*, whilst in other cereal

starches there is a significant amount of free fatty acids. If surface lipid contaminants are present they tend to be triglycerides derived from non-starch tissues. True starch proteins are located within the matrix of starch granules and probably represent entrapped biosynthetic enzymes. Surface contaminants and associated proteins may be present if the starch is improperly purified.

The amylose molecule is an essentially linear α-(1-4)-glucan. Whilst the structure is dependent on its genetic origin, in general the molecule comprises 2-11 chains with 100-700 glucose residues per chain and about 4,000 residues per molecule. This confers a molecular weight of about half a million. Amylopectin is a very branched molecule, containing about 5% α-(1-6) bonds (or 'branch points'). The unit chain lengths are relatively small, consisting of about 17-25 glucose residues. There are about 100,000 residues per amylopectin molecule giving a molecular weight of about 16.2 million. It is generally agreed that amylopectin consists of exterior chains, or A-chains, which are α-(1-6) bonded to internal chains called B-chains. Alternatively the A-chains may be directly bonded to the single 'backbone' or C-chain of the molecule which carries the only reducing group. The B-chains may be further α-(1-6) bonded to other B-chains or bonded to the C-chain. Adjacent exterior chains intertwine with each other as double helices held together by hydrogen bonding. These double helices adopt an ordered register and form concentric radiating crystalline shells extending from the centre, or hilum, of the starch granules to the periphery. Between the crystalline shells there are amorphous zones comprising α-(1-6) branch points and amylose. The amylose may be either free amylose (FAM) or lipid complexed (LAM).

In most normal cereal starches the apparent amylose content (determined colorimetrically by iodine binding before lipid extraction) of the α-glucan is about 20-25%, while the lipid extracted starches (where lipid accounts for about 1% of the starch mass) give a total amylose content of about 25-30%. Since non-cereal starches contain essentially no lipid, the apparent and total amylose contents are practically the same. Waxy cereal starches also contain very little lipid (except barley) and the apparent amylose content is comparable with the total amylose content, generally <5% amylose. High amylose cultivars, however, contain around 40-60% apparent amylose and 50-70% total amylose and the lipid content may be >1.5%.

The amylopectin fraction is the prime contributor to starch functionality. When starches are heated in excess water they gelatinise and swell. Gelatinisation involves uncoiling of the double helices and loss of crystallinity as the ordered register of these helices is lost. Crystalline regions become amorphous. This is reflected in loss of birefringence, crystallinity as quantified by wide angle X-Ray diffraction and number of double helices as quantified by ^{13}C-CP/MAS NMR. Some authors would contend that mechanically modified starches are similar to gelatinised starches in view of their comparable digestibility with amylases and amyloglucosidase. However, gelatinised starches have intact amylopectin molecules whereas in mechanically damaged starches amylopectin is fragmented. The onset (T_O), peak (T_P) and conclusion (T_C) temperatures of gelatinisation (determined by differential scanning calorimetry, DSC), and the associated enthalpy of the process (ΔH) are characteristic of the origin of starch. Typically T_O occurs at about 45°C, T_P at 60°C and T_C at 75°C for most starches.

Swelling is a progressive expansion process of starch granules that begins at around T_O. Assuming that water is not limiting (which is not always the case in foods) the granules continue to swell as a function of temperature in a fashion which is characteristic of the

botanical origin. Waxy starches swell more than normal starches and the latter swell more than high amylose starches. Normal tuber and root starches, for example, swell much more than cereal starches at a given temperature above the onset of swelling. This is partly due to LAM, which restricts swelling, whereas FAM facilitates it. The amylopectin of potato starch is also relatively heavily phosphorylated and this promotes swelling most probably because of phosphoester charge repulsion. However, the structure of amylopectin is the main determinant of the extent of swelling of granules. During swelling, α-glucan is leached as a function of temperature. In normal starches this is almost exclusively FAM.

The temperature-dependent swelling profile of many starches, between about 45 to 90°C, is either linear or includes a linear followed by a temperature-independent (*circa* 70 to 90°C) plateau region. At around 90°C the granules begin to disintegrate extensively, especially when a shear force is applied, and form a granule-free viscous colloidal dispersion (about 2% solids) or a gel structure (>2% solids). The changes of the viscosity of aqueous starch dispersions as a function of time and temperature are routinely characterised with a Brabender Amylograph. Starches are heated from 50 to 95°C (in a programmed fashion over 30 minutes) with slow stirring. As granules expand and interact with each other the viscosity increases rapidly. Leached α-glucan contributes also to the viscosity. If the starches are maintained at 95°C for 1 hour (with shearing) the viscosity decreases as the granular structure disappears due to the prolonged shear, and a colloidal dispersion is formed. Finally, upon cooling to 50°C and maintenance at this temperature, the viscosity begins to rise again as hydrogen bonding and the associated retrogradation process begin. The botanical source and composition of the starch have a marked effect on the rheological properties of the colloidal dispersions (pastes), and the characteristics of the gels, as well as the clarity of the resulting products (Table 2). These properties may be modified by chemical treatment as discussed below.

Table 2. Properties of gelatinised starches

Starch	Solution Viscosity	Gel Texture	Gel Clarity	Rate of Retrogradation	Freeze-thaw Stability	Resistance to Shear
Normal cereal	Medium	Rigid	Opaque	Rapid	Low	Medium
Normal potato, tapioca & sago	High	Soft	Clear	Rapid	Low	Low
Waxy cereal	Medium	Soft	Clear	Slow	Medium	Low
High amylose cereal	Low	Rigid	Opaque	Rapid	Low	High

We are currently investigating the significance of unit chain length, branching pattern, molecular weight and polydispersity in relation to swelling power in a broad range of starches; initial results indicate that the unit chain distribution exerts a critical control. A relatively high proportion of long (average DP *circa* 45-55) to short chains (average DP *circa* 15-25) would appear to enhance swelling and granule volume. The inverse would also appear to be true. In addition, we have been investigating the initiation of gelatinisation and swelling, which we believe begin in amorphous zones by destabilising crystallites, although not necessarily 'stripping starch chains from crystallites' as reported by Donovan (1979).

There are many features of starch where quality issues affect functionality. These can broadly be defined as:- (i) purity, (ii) composition, (iii) α-glucan structure, (iv) granule architecture, (v) environmental modification, (vi) chemical modification, (vii) enzymatic modification and, (viii) physical modification.

Purity is quite difficult to define. Industrially pure starch would mean free from surface contaminants and with a protein content of <0.5%, probably <0.3%. To attain this level of purity it is often necessary to use relatively high temperatures in conjunction with chemical treatment. For example maize is steeped at 45-50°C in aqueous SO_2 solution for at least 48 hours. This very effectively solubilises the proteins and softens the cellular tissues, but causes some annealing. In the laboratory we aim to remove the non-starch materials but recover the starch in its essentially natural state. To this end we inactivate amylases (usually with dilute acid), partially solubilise the proteins without allowing microbial growth (cold extraction in dilute sodium chloride and sodium metabisulphite solution and/or protease treatment) and remove residual non-starch materials from the heavy starch granules by centrifugation in an 80% solution of caesium chloride (high density separation). We also endeavour to extract the starch almost quantitatively and avoid loss of small granules. The extraction procedures we have developed achieve this successfully, although some loss of granules is inevitable. The presence of non-starch contaminants adhering to the surface of the granules will adversely affect the functionality of the starch as well as the purity of hydrolysates.

The composition of starch granules is under genetic control and essentially constant, although environmental conditions during growth of the plant can cause some modification. However the development of waxy and high amylose mutants has extended starch applications and improved the quality of many products. In recent years waxy potatoes and wheat have also been produced and the absence of amylose will hopefully promote novel applications. Probably of more interest now is the use of transgenic technology to alter the structure of amylopectin molecules and hence alter the crystallisation pattern and associated properties of the major functional component of starches. Using this approach, starches can be produced with properties which are 'tailor made' for given applications. These will include novel branching patterns and chain lengths, with associated variation in the site and extent of phosphorylation. This would reduce the need to modify starches chemically. Perhaps as biotechnology progresses it might be possible to insert sugar molecules other than glucose into 'starch' and create entirely novel and unique polymers.

The architectural aspects of starch granules, in terms of size and interactions within granules are largely under genetic control. We believe that interactions within starch granules are spontaneous and lead to the most thermodynamically favourable state. The exterior chains of amylopectin form double helices because this is favoured by their chain length and their location within the granule. The extent of ordering, or perfection of the crystalline register is, however, not necessarily optimised during biosynthesis and is subject to modification by environmental or processing conditions. Formation of amylose lipid complexes would also appear to be spontaneous during biosynthesis and their presence in native cereal starch granules has been demonstrated by NMR studies (Morrison *et al*, 1993). However, if the amount of lipid synthesised is altered the functionality of the starch will also be modified, because of the formation of LAM. Hence, factors that might cause variation should be characterised and suitably adapted by starch producers and processors alike to maintain product quality.

Environmental factors have a major impact on starch properties regardless of the botanical source. Work by ourselves on cereals (Tester *et al*, 1991 and 1995) and more recently tubers (unpublished data) shows quite definitively that growth temperature in particular has a marked effect on starch synthesis and properties. Elevating growth temperature from 10 to 20°C in wheat or barley tends to reduce granule size and number of granules per endosperm; to maintain a constant amylose content but increase lipid, and to cause an increase in T_P by about 1°C for every 1°C increase in growth temperature. It has, however, little or no effect on ΔH. Increasing the growth temperature above the optimum for potatoes similarly reduces starch granule size and increases T_P. We describe this as *'in vivo* annealing' and attribute it to perfection of crystalline register and ordering of double helices within crystallites. It is very important, therefore, that starch manufacturers and food processors are aware of the environmental history of the product as a very large seasonal variation in functionality can occur.

Food processors demand certain functional properties from starches. Since at present native starches cannot meet these, a number of different types of modification are performed by starch producers to meet the demand and product quality specifications. Chemical modifications are designed to achieve different aims. Acid hydrolysis, for example, is used to produce soluble starch and was traditionally used extensively to produce glucose syrups from starch. Gelling/thickening characteristics can be improved to resist heat and freeze-thaw instability, shear thinning and low pH effects in food products. This may incorporate cross-linking, esterification, etherification and oxidation alone or in combination.

Enzymatic modification is now widely used by glucose syrup manufacturers in preference to acid hydrolysis. Starches are heated in the presence of α-amylase to produce a number of products with varying proportions of sugars, oligosaccharides and dextrins. Usually amyloglucosidase is used to produce high dextrose equivalent (DE) products with a low proportion of dextrins. Sometimes β-amylase is used in preference to amyloglucosidase to produce high maltose syrups, which are more commonly used in the US than Europe. Starches that have been treated with α-amylase, even at very low levels of conversion, have reduced swelling properties because of critical cuts in the amylopectin molecules. Hence it is crucial that in relation to starch quality enzymatic modification does not occur in the field or prior to starch extraction. During wet seasons where grain may germinate before harvest the quality will suffer as a consequence of amylase hydrolysis with detrimental affects on baking quality. It is sometimes assumed that during malting there is a high level of starch conversion to sugars. In fact, whilst the starch would not be appropriate for use as a functional ingredient because of the modification, there is only a relatively small proportion of sugars in the grain, normally a few percent.

Mechanical damage produces starches with unique swelling and leaching characteristics, which also reflect the botanical origin of the starch. A number of fractions are produced when native granules are mechanically damaged and have been described by Morrison *et al* (1994) as native granules, ordered/birefringent granule fragments, cold water swelling granule fragments and soluble amylopectin fragments. Traditionally damaged starch has been considered to be a problem and have a negative impact on starch quality. In partnership with industry, we are now beginning to understand what the commercial benefits might be in relation to the usage of damaged starch in novel applications, and these results look very promising.

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APPENDIX B

Swelling and Gelatinization of Cereal Starches. I. Effects of Amylopectin, Amylose, and Lipids[1]

RICHARD F. TESTER and WILLIAM R. MORRISON[2]

ABSTRACT

A method was developed for measuring the volume of water absorbed by starch granules heated in excess water, based on the observation that blue dextran dye (molecular weight 2×10^6) will dissolve in supernatant and interstitial water but not in the intragranular water. Swelling curves of wheat and normal and waxy barley and maize starches, determined by measuring the swelling factor (swollen volume/initial volume of air-dried starch) at various temperatures up to 85°C, were characterized by an initial phase of slight swelling, a second phase of rapid swelling, and a final stage of maximum swelling (not observed with high-gelatinizing starches or if granules disintegrated). With wheat starch, swelling began at 45-50°C and continued to 85°C; loss of birefringence and a large decrease in gelatinization enthalpy attributed to dissociation of crystalline clusters occurred at 50-55°C, and residual enthalpy attributed to dissociation of double helices was lost at 55-60°C. With all starches, leaching of polysaccharide (amylose and/or amylopectin, depending on the starch) was highly correlated with swelling factor. Experiments with waxy and normal starches lead to the conclusion that swelling is a property of the amylopectin. In normal cereal starches, amylose and lipids actively inhibit swelling, except in barley starch above 60°C where they only act as diluents. Characteristic buckling of lenticular A-granules from wheat and barley (waxy and normal types) is attributed to preferential swelling and leaching of polysaccharide at the equatorial groove, where there is less amylose and lipid and where the amylopectin appears to be less crystalline.

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Gelatinization in the narrowest sense is the thermal disordering of crystalline structures in native starch granules, but in the broader sense it includes related events such as swelling of the granules and leaching of soluble polysaccharides (Atwell et al 1988). Gelatinization temperature (GT) and enthalpy (ΔH) are conveniently measured by differential scanning calorimetry (DSC), and this aspect has received much attention in recent years because it is experimentally convenient and precise.

However, in most food systems the actual temperature at which starch gelatinizes is less important than those properties that depend on swelling, such as pasting behavior and rheological properties of the partially or fully swollen starch granules. The properties of the starch-water system will, of course, be different if the swollen granules are dispersed mechanically to give a uniform gel.

Historically, starch swelling has been studied by simple methods that do not distinguish between intragranular water and intergranular or interstitial water (Leach et al 1959), and the precision of measurements was not particularly good. This paper describes an improved method for measuring only intragranular water and hence the true swelling factor at a given temperature, based on the observation that blue dextran (M_r 2×10^6) does not penetrate swollen granules. The effects of amylopectin (AP), amylose (AM), and lipids on swelling behavior were then investigated using the blue dextran method.

MATERIALS AND METHODS

Starches

Wheat, barley, or maize grain (5-100 g), cracked by passing between smooth rolls set to an appropriate gap, was steeped in water at 3-5°C for 1-3 hr, then ground gently to release a suspension of starch that was passed through a 75-μm aperture sieve. The crude starch was recovered by centrifuging (1,550 $\times$ g, 15 min), slurried in a small volume of water, layered above 30 ml of 80% (w/v) CsCl in 70-ml tubes, and centrifuged at 30,000 $\times$ g for 20 min at 15°C. This procedure was repeated twice and the starch was then washed six times with water, centrifuging for 5 min at 1,550 $\times$ g to recover starch at each stage. The starch was air-dried to give a free-flowing powder. Centrifuging through 80% cesium chloride removes most proteins associated with the surfaces of starch granules (Sulaiman and Morrison 1990).

Wheat starch was size-fractionated by sedimenting through 18 cm of water at 5°C for various times (Decker and Höller 1962, Morrison and Gadan 1987).

Physical Measurements

Dimensions of native granules and of partially swollen granules were measured using a Coulter Counter with 100-channel analyzer (Morrison and Scott 1986). DSC determinations of GT and ΔH of the major endotherm attributed to disordering of AP were made on triplicate samples (3-4 mg of starch, 15 μl of water) heated from 5 to 100°C at 10°C/min (Soulaka and Morrison 1985). T_o, T_p, and T_r are the onset, peak, and recovery (return to baseline) temperatures of the endotherm. No measurements were made on the AM-lipid endotherm in the region 94-120°C.

For scanning electron microscopy (SEM), one drop of a suspension of starch (native or partially swollen) was placed on a filter paper with electrically conducting adhesive, and frozen with liquid nitrogen within an EMScope SP2000 sputter cryo-system (EMScope), then etched at −65°C for 10 min and sputter coated with gold at 0.15 torr in an argon atmosphere. The samples were then examined in a Jeol T200 (JEOL) scanning electron microscope with an accelerating voltage of 2-5 kV.

Chemical Analyses

Moisture content was taken as weight loss after heating at 130 ± 3°C for 1 hr. Starch lysophospholipid content was obtained by multiplying phosphorus content (Morrison 1964) by the factor 16.5 (Morrison et al 1975). Starch lipids were obtained by extraction with propanol-water (3:1, v/v) at 100°C (Morrison and Coventry 1985) for methanolysis and quantitative gas chromatography (Morrison et al 1975, 1980).

Wheat starch with its lipids partially extracted was obtained by heating 1-g samples with 8 ml of anhydrous methanol under nitrogen at 100°C for 6 hr, discarding the extracts, repeating the extraction twice, then air-drying the starch. Controls consisted of 1 g of starch heated in 1 ml of methanol for 18 hr, which was then evaporated in the tube under a stream of nitrogen so that no lipids were removed.

Total starch (α-glucan) was determined as glucose (×0.9) by the method of Karkalas (1985). Soluble starch was determined similarly, omitting the initial α-amylase digestion prior to conversion of dextrins to glucose with amyloglucosidase. Amylose was determined colorimetrically on lipid-free starch (obtained by precipitating from urea-dimethylsulfoxide solution with ethanol) by the method of Morrison and Laignelet (1983). Starches were debranched with isoamylase and the linear α-1,4-glucan chains separated by gel permeation chromatography on a column of

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Sepharose CL-6B (Morrison et al 1984). Native starches were similarly fractionated on a column of Sepharose CL-2B.

Determination of Swelling Factor

Direct method. Starch (50, 100, or 200 mg, depending on anticipated swelling factor) was weighed correct to 0.1 mg into replicate 10-ml screw-cap tubes, 5.0 ml of water added, and the sealed tubes incubated with constant shaking in a waterbath at the required temperature for 30 min. The tubes were then cooled rapidly to 20°C, 0.5 ml of blue dextran (Pharmacia, M_r 2×10^6, 5 mg/ml) was added, and the contents mixed by gently inverting the closed tubes several times. After centrifuging at $1,500 \times g$ for 5 min the absorbance of the supernatant (A_S) was measured at 620 nm. The absorbance of reference tubes (A_R) that contained no starch was also measured.

Calculation of swelling factor (SF) was based on starch weight corrected to 12% moisture, assuming a density of 1.4 g/ml. Free or interstitial-plus-supernatant water (FW) is given by

$$FW(ml) = 5.5\,(A_R/A_S) - 0.5 \quad (1)$$

the initial volume of the starch (V_0) of weight W (in milligrams) is

$$V_0\,(ml) = W/1{,}400 \quad (2)$$

and the volume of absorbed intragranular water (V_1) is thus

$$V_1 = 5.0 - FW \quad (3)$$

hence the volume of the swollen starch granules (V_2) is

$$V_2 = V_0 + V_1 \quad (4)$$

$$\text{and } SF = V_2/V_0 \quad (5)$$

This can also be expressed by the single equation

$$SF = 1 + \{(7{,}700/W)[(A_S - A_R)/A_S]\}$$

The coefficient of variation of the method was generally less than 1%.

Indirect method. After incubating samples in the waterbath and cooling to 20°C, 1 ml of hexadecane/carbon tetrachloride (53:57, w/w) was added and the tubes centrifuged at $2,500 \times g$ for 15 min to give a starch gel layer below the solvents and free water above the solvents. Blue dextran (0.5 ml) was added to the top layer, which was gently stirred with a rod (without disturbing the solvent layer), and an aliquot was withdrawn to determine A_S or A_R.

For starch samples swollen in the presence of substances that affect blue dextran (e.g., acids) or that cause turbidity (e.g., emulsifiers), 1.0 ml of 0.2*M* NaCl was added instead of blue dextran, and an aliquot of the top layer was titrated with 0.01*M* $AgNO_3$ using chromate indicator. The calculations of FW and SF were amended accordingly. The indirect method was not used in the studies described in this paper, but is included here for completeness.

TABLE I
Effect of Water/Starch Ratio on Swelling Factor at 70°C and on the Gelatinization Endotherm of Wheat Starch Measured by DSC[a]

Water/Starch Weight Ratio	Volume Fraction of Water	SF_{70}	T_o (°C)	T_p (°C)	T_r (°C)	ΔH (J/g)
1:1	0.58	2.56	49.9	56.8	67.8	6.2
2.5:1	0.78	3.56	47.1	57.9	72.4	11.9
5:1	0.88	6.01	48.5	58.0	72.2	11.2
7.5:1	0.91	6.25	47.5	58.1	72.3	11.2
10:1	0.93	6.68	48.8	58.2	71.1	10.3
20:1	0.97	7.01	51.9	58.2	69.6	10.1

[a] Differential scanning calorimetry.

RESULTS AND DISCUSSION

Development of SF Method

When wheat starch was incubated at 60, 70, and 80°C for various times, there was a period of rapid swelling lasting 5–10 min, followed by further small increases up to 5 hr, and a different swelling curve was obtained for each temperature. Although complete equilibrium had not been reached, SF measured at 30 min was chosen for convenience in all subsequent studies. SF was not affected by presoaking the starches before heating, and it did not change once the heated starches had been cooled. SF increased with the water/starch ratio over the range 0.1–2.0 ml of water/100 mg of starch, but was nearly constant with >2.5 ml of water/100 mg of starch (water/starch = 25:1). Thus starch samples in the range 50–200 mg could be used for determination of SF, the smaller samples being necessary when SF was large.

Swelling and Gelatinization of Wheat Starch

It is well known that when starches are heated in progressively limited amounts of water, the DSC thermograms show decreases in the so-called gelatinization endotherm and the appearance of other endothermic peaks at higher temperatures (Donovan 1979, Wootton and Bamunuarachchi 1979, Blanshard 1987). The gelatinization endotherm is attributed to disordering of AP crystallites and is quite distinct from endotherms due to dissociation of retrograded AM or of AM-lipid complexes (Stute and Konieczny-Janda 1983, Morrison 1988a).

When swelling factors were calculated from granule volumes measured with a Coulter counter, they remained constant (1.0) up to 45°C, then increased to 1.7 at 65°C and declined at higher temperatures as the swollen granules became almost completely permeable to electrolyte ions. The discrepancy between the blue dextran and Coulter swelling factors at all temperatures much above the onset of swelling shows that very little swelling is needed to cause electrolyte ion conduction through the swollen granules. Hence, the Coulter method, which is normally used to measure the volume of native granules, can also be used to detect the onset of swelling.

Parallel measurements of SF and the gelatinization endotherm were made on wheat starch (Table I). The DSC thermograms showed the expected pattern, with a single sharp endotherm only when the volume fraction of water exceeded 0.7. However, swelling was obviously very incomplete at this level of water, and maximum swelling at 70°C (close to T_r) was only achieved with a volume fraction of 0.97 (water/starch ratio = 20:1). Even allowing for the fact that SF is measured under conditions nearer to equilibrium than in DSC, the discrepancy does suggest that swelling at 70°C involves more changes than were measured by the DSC endotherm.

Figure 1 shows a complete swelling curve for wheat starch isolated from a soft European wheat. SF values above 85°C were not obtained because the granules began to disintegrate. Table II gives DSC measurements on starch preincubated to various temperatures as for determination of SF. Swelling began at 45–50°C, coinciding with the onset of gelatinization measured by DSC (T_o = 45–50°C) but well before the peak temperature (T_p = 58°C). Loss of birefringence at 50–55°C coincided with the first decreases in enthalpy (45–55°C), but further decreases were observed at 57.5 and 60°C. Swelling then continued to increase linearly up to 85°C, well above the temperature at which order detectable by DSC was no longer observed.

Our interpretation of these events is as follows. In the native granules crystalline order is found in clusters of double helices formed by adjacent external chains of AP. Dissociation of clusters and loss of birefringence with a substantial change in enthalpy occurred at 45–55°C. From 55–60°C there was a further change in enthalpy due to dissociation of double helices (which were not birefringent). Above 60°C it is postulated that the external chains have a restricted semirandom conformation—restricted because the existence of swollen granules requires some intermolecular (hydrogen?) bonding, and semirandom because no order was detectable.

It is well known that some starch is solubilized by leaching when granules gelatinize. In this study, polysaccharides leached from undamaged granules over the range 50–85°C were examined (Fig. 1). Amounts of leached total α-glucan and AM were very highly correlated ($P < 0.001$) with the extent of starch swelling from 60 to 80°C, which suggests a strong interdependence. Lindqvist (1979) has shown that AM leaching is a prerequisite for the cold-gelatinization of starches induced by electrolytes. Total α-glucan increased from 0.2 mg/100 mg of starch at 50°C to 7.9 mg/100 mg of starch at 85°C, whereas AM (measured colorimetrically) showed an almost parallel increase from zero at 50°C to 7.3 mg/100 mg of starch at 85°C. Gel permeation chromatography of the α-glucan leached at 70°C (3.7 mg/100 g of starch, containing approximately 90% AM) showed that it contained about 10% material eluting at the void volume (ahead of AM), consistent with it being AP. Since this starch had a low level of damage, this may have been cold water-soluble fragments of AP (Craig and Stark 1984, Stark and Yin 1986, Yin and Stark 1988), and, within experimental error, it accounted for the nearly constant difference between α-glucan and apparent AM at all temperatures.

Previous studies have shown that the α-glucan leached at lower temperatures is lower molecular weight linear AM and at higher temperatures it is higher molecular weight branched AM with starches from potato (Cowie and Greenwood 1957a, 1957b), barley (Banks et al 1959), amylomaize and pea (Banks and Greenwood 1975), and wheat (Ghiasi et al 1982). However, oat starch leaches AM and AP together at all temperatures (Doublier 1981, Doublier et al 1987), and waxy starches (discussed later), which have almost no AM, leach AP.

There was no detectable lipid in the leached polysaccharides. This was to be expected, since AM-lipid complexes are insoluble in water and do not dissociate unless heated above 94–98°C (Morrison 1988a, Raphaelides and Karkalas 1988). However, inclusion complexes may have been formed between the natural starch lipids and the residual AM in the granules during swelling (Morrison 1988b), and this would have prevented that fraction of the AM from leaching. The starch contained enough lipid to form lipid-saturated complexes (Karkalas and Raphaelides 1986) with 7–8% AM in the starch (total AM was 29%), hence the maximum AM that could leach would be about 20% of the total starch. In practice, only 7% AM leached from the starch in 30 min at 85°C, and little more was recovered when swelling was continued for 5 hr, hence some restraint other than insolubilization by lipid has to be considered. When AM complexes with lipids in solution it is an all-or-nothing process giving lipid-saturated complexes and free AM when lipids are limiting (Karkalas and Raphaelides 1986). However, steric restraints within gelatinizing starch granules could enable partially filled complexes to be formed. The solubility properties of such complexes, if they exist, could be sufficient to prevent AM leaching.

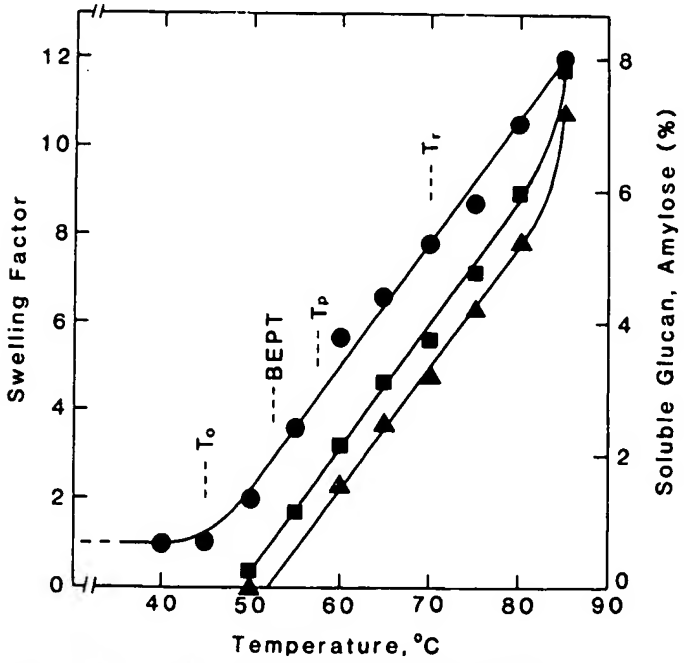

Fig. 1. Swelling curve of starch from a soft European wheat and amounts of polysaccharide (■) and amylose (▲) leached from the starch when heated in water for 30 min at temperatures up to 85°C.

Effects of Amylopectin, Amylose, and Lipids

In cereal starches AM content is often correlated with lipid content (Morrison 1988b), and it is difficult to distinguish the effects of each on granule swelling and gelatinization. Furthermore, selecting starches from unrelated varieties of the same cereal or from different species of cereals is likely to introduce considerable variation attributable to differences in AP, which makes it impossible to interpret the results satisfactorily. Comparisons were therefore made between waxy barley and maize starches (essentially pure AP) and their near-isogenic normal counterparts to establish the contributions to gelatinization and swelling behavior of AP on the one hand and of AM plus lipid on the other.

In the first experiment, starches from a normal barley (Oderbrucker) and its waxy mutant (Waxy Oderbrucker) were used. Being near-isogenic, it is probable that AP in both would be very similar. This is supported by the DSC data (Table III), which show almost identical gelatinization temperatures. If AM and lipids were merely diluents as far as gelatinization (measured by DSC) is concerned, enthalpy calculated on the basis of AP content rather than on starch weight would be comparable, but it was slightly higher for the normal starch (14.5 J/g) compared with the waxy starch (12.7 J/g), indicating small differences in AP crystalline order.

Since the waxy barley starch swelled much more than the normal starch (Fig. 2), it would seem that swelling is primarily a property of AP. The normal Oderbrucker swelling curve was therefore recalculated on the basis of AP content and effectively coincided with the Waxy Oderbrucker curve from 60°C upwards, which shows that AM and lipids were only acting as diluents at this stage. Both starches had evidently reached maximum swelling

TABLE II
Differential Scanning Calorimetry Gelatinization Endotherm of Wheat Starch Swollen by Incubating in Excess Water at Various Temperatures for 30 min

Incubation Temperature (°C)	Swelling Factor	T_o (°C)	T_p (°C)	T_r (°C)	ΔH (J/g)
30	1.0	50.0	58.1	71.1	10.7
35	1.0	46.0	57.8	75.0	11.3
40	1.0	50.0	58.1	76.0	11.3
45	1.0	49.0	58.9	74.5	11.2
50	2.3	53.5	61.8	75.0	7.5
55	3.8	60.0	65.5	72.5	1.5
57.5	4.4	59.5	68.1	74.5	1.3
60	5.1	...[a]	71.9	...[a]	...[a]
>60	>5.1	NE[b]	NE	NE	NE

[a] Differential scanning calorimetry of the ungelatinized starch gave a very small endotherm and a T_p value but no other reliable measurements.
[b] No endotherm.

TABLE III
Amylose Content and Gelatinization Properties of Normal and Waxy Starches from Barley and Maize

Starch	Amylose (%)	T_o (°C)	T_p (°C)	T_r (°C)	ΔH (J/g)
Barley					
Normal	27.5	46.7	56.5	73.7	10.5
Waxy	5.6	43.7	57.6	77.0	12.1
Maize					
Normal	29.4	58.3	70.7	83.0	8.7
Waxy	3.0	60.8	72.4	85.3	14.5

factor by 70°C. This feature was not observed in the wheat or maize starches under the conditions used here, but was confirmed with six low-GT rice starches (Tester and Morrison 1990).

With both starches swelling began at about 40°C, close to T_o (Table III) and coinciding with the point when leaching of polysaccharide began (Fig. 2). The polysaccharide leached from normal Oderbrucker starch was mostly AP at 40 and 50°C, but progressively more AM was leached at higher temperatures, together with some AP. The Waxy Oderbrucker starch leached pure AP, although the starch did contain 5.6% AM, and thus behaved differently from waxy maize starch (below). The reason for the decrease in AP leached at 80°C is not known.

A similar experiment was done using near-isogenic lines of normal and waxy maize (Morrison et al 1984). Gelatinization temperatures were nearly identical (Table III), but the enthalpy of the normal starch recalculated on the basis of AP content (12.3 J/g) was less than that of the waxy starch (14.9 J/g). Swelling of the waxy starch began at about 55°C, but the real inflection point in the curve was at 60°C (Fig. 3) coinciding with T_o (Table III). Leached polysaccharide (which was almost entirely AP) increased from 1.5 mg/100 mg of starch to 18.6 mg/100 mg of starch at 80°C and closely paralleled the swelling curve. Compared with the Waxy Oderbrucker starch, the waxy maize starch leached 10 times more AP from T_o to T_p.

The normal maize starch gave a low swelling curve starting from 50–55°C (Fig. 3) with a linear increase in leached polysaccharide (AM) from 0.4 mg at 50°C to 6.0 mg/100 mg of starch at 80°C, comparable with the wheat starch. The polysaccharide leached at 50°C was AP, and AM together with some more AP was leached at higher temperatures, comparable with the barley starch but not the wheat starch. The swelling curve recalculated on the basis of AP content was far below that of the waxy starch, showing that AM and lipids in the granule strongly inhibited swelling at all temperatures.

To examine the effects of natural starch lipids alone (as opposed to added lipids such as the AM-complexing surfactants) solvent extraction of lipids was used. Efficient extraction with hot alcohol-water mixtures causes controlled swelling of the starch granules (Morrison and Coventry 1985) which was unacceptable here, but hot anhydrous methanol is a reasonably efficient solvent and has little effect on GT and ΔH (Raphaelides 1986), so it may be presumed to cause little disturbance to AP crystallites.

Ten wheat starches were partially extracted with methanol at

TABLE IV
Composition, Swelling (at 70°C), and Gelatinization Properties[a] of 10 Wheat Starches Before and After Partial Extraction of Lipids with Anhydrous Methanol at 100°C

Property	Before	After
Amylose content (%)	29.2 (0.7)	29.2 (0.7)
Lipid content (mg %)	852 (64)	457 (83)
Gelatinization temperature (°C)	57.9 (1.5)	57.4 (1.3)
Swelling factor		
Native starch	7.7 (0.3)	...
Extracted starch	8.4 (0.3)[b]	11.0 (0.8)

[a] Mean values with standard deviations ($n = 10$).
[b] Methanol-treated without removal of lipids (described in Methods).

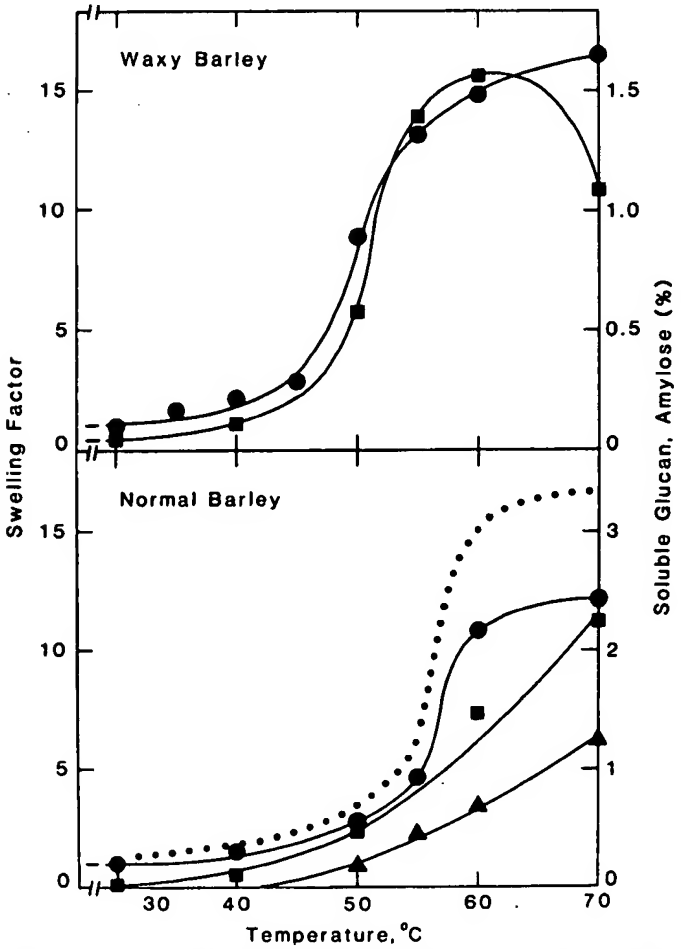

Fig. 2. Swelling curves of starches from normal barley (Oderbrucker) and waxy barley (Waxy Oderbrucker) (●), and amounts of polysaccharide (■) and amylose (▲) leached from starches when heated in water for 30 min at temperatures up to 70°C. Dotted line shows swelling factor of normal starch recalculated on basis of amylopectin content.

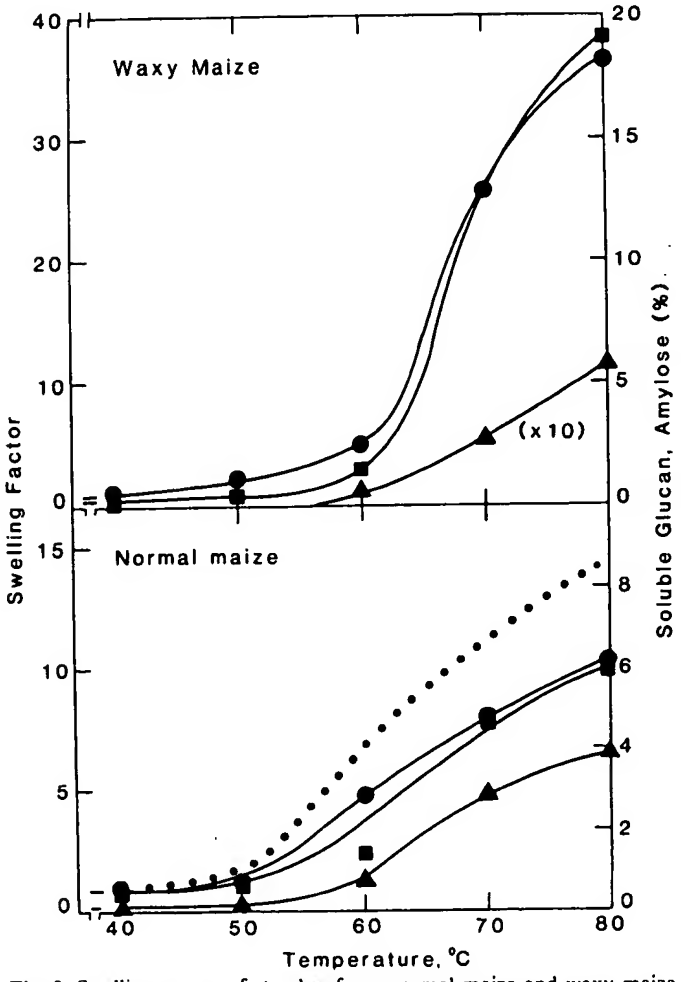

Fig. 3. Swelling curves of starches from normal maize and waxy maize (●), and amounts of polysaccharide (■) and amylose (▲) leached from starches when heated in water for 30 min at temperatures up to 80°C. Dotted line shows swelling factor of normal starch recalculated on basis of amylopectin content.

100°C, and their swelling and gelatinization properties were determined before and after extraction (Table IV). All properties of the native starches exhibited a small range of variation, but there were no significant correlations between any pairs of parameters. Methanol extraction removed about half the lipid and had a negligible effect on GT and ΔH. However, unextracted methanol-treated starches showed a small increase in SF (av. 0.7) probably caused by disturbing some of the lipids that would have been extracted then redeposited on the surface of the granules when the methanol was evaporated. Taking this into account, the effect of removing half the starch lipids was to increase SF by 30%, from an average of 8.4 to 11.0.

From this experiment, it was concluded that the natural lipids in wheat starch caused a substantial suppression of swelling at 70°C and probably at all points on the swelling curve up to 85°C. Results from similar studies using extraction with hot aqueous methanol cannot be compared too closely because the starches were partially gelatinized (Goering et al 1975, Lorenz 1976, Melvin 1979, Lorenz and Kulp 1983).

Wheat starch separated into size fractions by sedimentation offered a further opportunity to study the effects of AM and lipids, since starch AM content decreases and lipid content increases as granule size decreases in mature starches (Morrison and Gadan 1987). Four fractions sedimenting at 0.25, 0.5, 1.0, and 1.5 hr were lenticular A-granules containing 28.4–27.8% AM and 674–731 mg/100 g of lipid. Three fractions sedimenting at 2, 5, and 16 hr were B-granules containing 27.5–24.5% AM and 730–909 mg/100 g of lipid. T_p increased as granule size decreased from 57.3°C (0.25–1 hr fractions) to 57.7–58.2°C (1.5–5 hr fractions) and to 60°C in the 16-hr fraction. Enthalpy (ΔH) was 11.5–12.0 J/g in the large A-granules (0.25–1.0 hr fractions) and 10.5–11.0 J/g in the other fractions. Thus the gelatinization endotherms measured by DSC were very similar, except for the 16-hr fraction, where the high T_p may have been an artifact.

The swelling factor of the A-granules (7.1 ± 0.2) was 25% greater than that of the B-granules (5.7 ± 0.1) despite the latter having slightly more AP which should have enhanced SF. Since the B-granules had more lipid than the A-granules, this suggests that lipid was responsible for the differences in swelling factor (mediated through AM) rather than the AM itself.

SEM of Swollen Starch Granules

Wheat starch A-granules develop asymmetrically (Evers 1971), and their AM and lipid contents increase up to maturity while the number of A-granules per endosperm remains constant (Morrison and Gadan 1987). From this, it has been deduced that the granules have a low-AM low-lipid interior and a high-AM high-lipid exterior, particularly towards the faces above and below

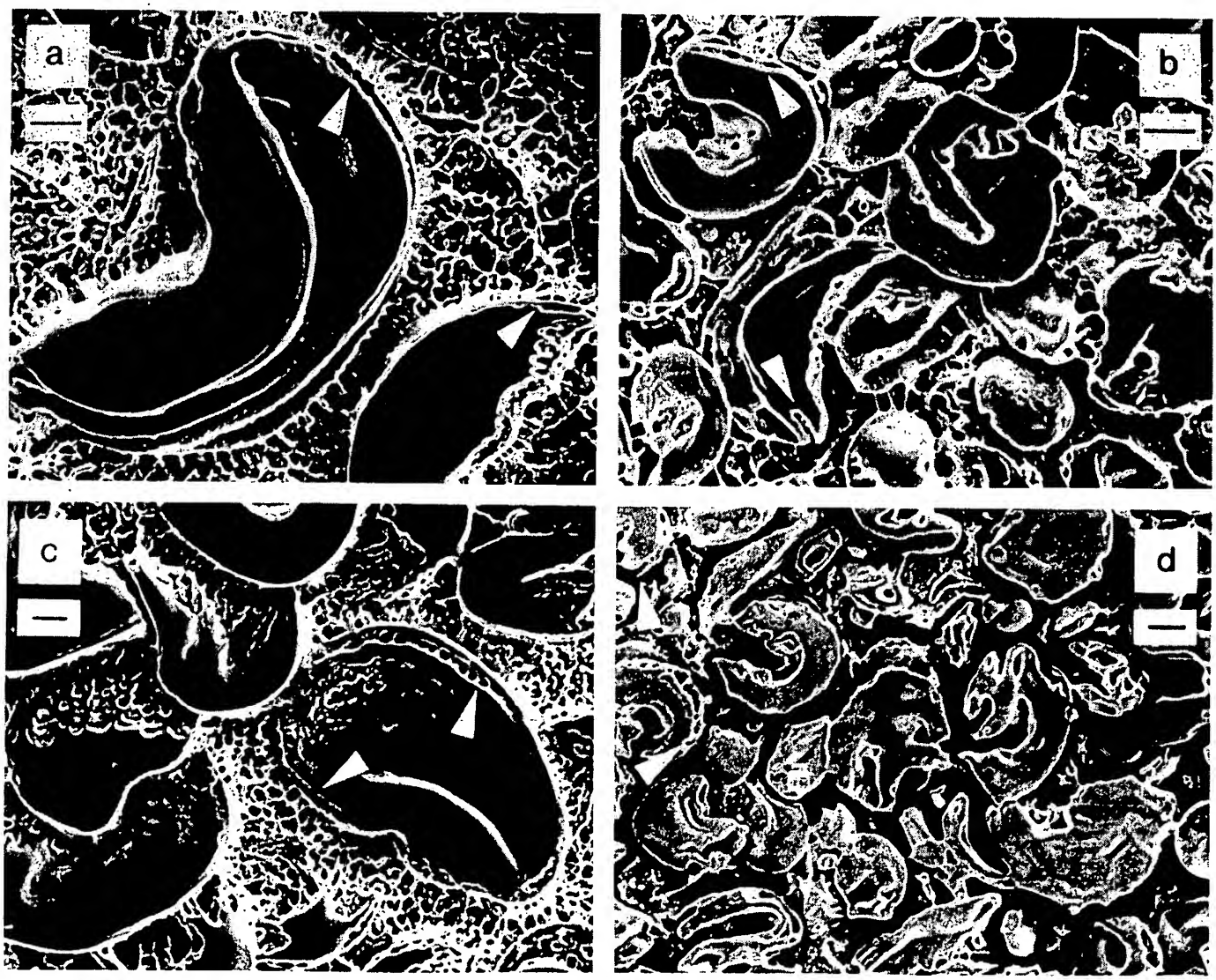

Fig. 4. Freeze-etch scanning electron micrographs of A-type wheat and barley starch granules. Granules from wheat heated in water at 70°C for 30 min (a and c) and from Waxy Oderbrucker barley starch heated in water at 54°C for 30 min (b and d). Arrows indicate pores in equatorial groove regions (described in text). Note substantial interstitial matrix of leached amylose from wheat starch (a and c) and comparatively little leached polysaccharide from waxy barley starch (b and d). Scale bars represent 10 μm.

the equatorial groove (Morrison 1989). When wheat starch is heated in water, the A-granules exhibit characteristic swelling mostly in the equatorial plane, and at 50–70°C they buckle into characteristic "saddle" shapes (Hoseney et al 1977, Bowler et al 1980). This behavior was confirmed in the present study (Fig. 4a) and is consistent with the compositionally asymmetric model of the A-granule and with results presented above that show that higher levels of AM and lipids will retard swelling.

Recent work with starches from developing barley show very similar patterns of change in the A-granules (McDonald et al 1989), which also buckle on heating (Williams and Bowler 1982), and this can be explained in the same way. However, it was found using light microscopy and SEM that Waxy Oderbrucker A-granules also swelled and buckled in an identical manner over the range 50–65°C (Fig. 4b). Since the AM content of Waxy Oderbrucker A-granules increases from 1.4% when immature to 5.8% at maturity, and lipid content increases from 75 to 385 mg/100 g at the same time (McDonald et al 1990), there might be enough AM and lipid to suppress swelling perpendicular to the equatorial plane. The alternative explanation, which seems more probable, is that there was asymmetry in the distribution of AP molecules (structure and crystallinity) so that those in the cheek regions (deposited later) swelled less than those in the equatorial plane (deposited earlier). Wheat A-granules are highly birefringent viewed end-on but are weakly birefringent when viewed perpendicular to their equatorial plane (Blanshard 1987). Since wheat and barley starches are morphologically similar, it may be assumed that normal and waxy barley A-granules exhibit the same anisotropy, which causes preferential swelling in the less crystalline equatorial plane. The explanation above does not exclude the distinct possibility that leaching of polysaccharide, intimately associated with the swelling of normal and waxy granules (Figs. 1–3), occurred preferentially at the equatorial groove that marks the exterior of the plane. Close examination of numerous micrographs of wheat and barley A-granules at advanced stages of swelling showed concentrations of pores in the equatorial groove (Fig. 4a–d). Such features can be readily dismissed as artifacts of sample preparation and drying procedures (Bowler et al 1987), although this is less likely with the freeze-etching technique used here. Random porous structures observed in the amorphous material surrounding extensively swollen granules were undoubtedly artifacts. It is the authors' opinion that the pores (whether artifactual or real) seen in the equatorial groove of partially swollen A-granules from wheat and from normal and waxy barleys are good evidence of preferential leaching of polysaccharide at these sites, and that this relates to their swelling and buckling behavior.

The composition of B-granules in wheat and barley, and of maize starch, also change during grain development (Shannon and Garwood 1984, Morrison and Gadan 1987, McDonald et al 1990), but there is no evidence for asymmetric deposition of starch polysaccharides or lipids, or for anisotropic distribution of crystallites (Blanshard 1987). SEM of these starches at several stages of swelling showed that expansion was essentially uniform until the granules were near total disruption, indicating an isotropic structure.

CONCLUSIONS

The experiments described in this paper showed that the swelling of cereal starch granules heated in water was associated with a sequence of events, notably disordering of crystalline structures, which could be followed by loss of birefringence and the DSC *G* endotherm, followed by further temperature-dependent swelling to reach a maximum swelling factor in the case of the barley starches and low-GT rice starches (described in the companion paper, Tester and Morrison 1990). Swelling is evidently a property of AP, and AM is thus a dilutent. However, AM and lipids in the normal starches also inhibit swelling under conditions when AM-lipid complexes are likely to be formed. Polysaccharide (AM, AP, or both, depending on the starch) leached from the granules is generally highly correlated with the extent of swelling for each starch. Wheat and barley A-type granules are lenticular and chemically and physically asymmetric (anisotropic) with higher levels of radially ordered crystallites and higher levels of AM and lipids in the cheek regions perpendicular to the equatorial plane. All these factors would act in the same way to reduce swelling in this region compared with the less crystalline, low-AM and low-lipid equatorial plane.

Clearly, many factors can contribute to swelling and gelatinization behavior, and a simpler system for study would be desirable. The companion paper (Tester and Morrison 1990) describes similar studies with low-GT and high-GT waxy rice starches that were essentially pure AP.

ACKNOWLEDGMENTS

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APPENDIX C

Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats[1,2]

Jörgen Holm, MSc; Ingmar Lundquist, MD, PhD; Inger Björck, PhD; Ann-Charlotte Eliasson, PhD; and Nils-Georg Asp, MD, PhD

ABSTRACT Glycemic response after ingestion of starchy foods varies. Starch in many common ready-to-eat foods is only partly gelatinized. In view of this, the relationships among degree of starch gelatinization, in vitro digestion rate, and in vivo metabolic response in rats were studied. Wheat starch with different degrees of gelatinization was used in the experiments. Plasma glucose and insulin responses as well as the rate of in vitro hydrolysis with α-amylase were strongly correlated to the degree of starch gelatinization ($r = 0.88$, $r = 0.90$, and $r = 0.96$, respectively). Plasma glucose and insulin responses were also positively correlated to the rate of hydrolysis with α-amylase in vitro ($r = 0.98$ and $r = 0.76$, respectively). These results suggest that the degree of starch gelatinization is an important determinant both for the rate of starch hydrolysis in vitro and for the metabolic response in vivo. *Am J Clin Nutr* 1988;47: 1010–6.

KEY WORDS Starch gelatinization, starch digestion rate, α-amylolysis, plasma glucose, plasma insulin

Introduction

The variable glycemic response after ingestion of starchy foods has been the subject of much interest recently, especially in relation to diabetes. So far, however, not much attention has been paid to the importance of the degree of starch gelatinization (DG).

In plant cells starch is present as granules. Starch polymers (amylose and amylopectin) are tightly packed in granules with a high degree of molecular order and are associated by hydrogen bonding. Raw granules contain highly crystalline regions and are birefringent in polarized light. The granules are insoluble in cold water. When exposed to heat in the presence of water, the starch granules undergo an irreversible swelling and destruction of the internal crystalline structure and birefringence is lost. This transformation is termed gelatinization. With excessive treatment the granules may even rupture and disintegrate and a fraction of the starch is then solubilized.

Raw starch is only slowly digested by enzymes in vitro whereas cooking increases the susceptibility considerably because of the rupture and disintegration of the compact crystalline granular structure (1–4). Furthermore, the glucose and insulin responses in vivo are significantly greater after ingestion of cooked compared with raw starches (3–7). Consequently, DG is an extremely important factor in the rate of starch hydrolysis and metabolic response.

However, in many common plant foods the starch is only partly gelatinized, because of the limited water content during processing. The starch granules are only slightly swollen and the internal structure is partly intact. Examples of such foods are breakfast flakes of cereal grains (3) and several baked products (8–11). The starch granules in legumes may swell incompletely during processing (12, 13). In view of the increased attention to starchy foods and the nutritional advantages of carbohydrates that are slowly digested and absorbed (14–19), the nutritional properties of incompletely gelatinized starch are of interest.

This paper describes the close relationship between the DG of pure wheat starch and the enzymatic susceptibility in vitro as well as the glucose and insulin responses to starch in rats. The results are discussed in terms of a

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TABLE 1
Physical and morphological characteristics of wheat-starch samples used in the experiments*

Treatment temperature†	Heat of gelatinization [n]	Degree of gelatinization: Calorimetric method (DSC) [n]	Degree of gelatinization: Enzymatic method [n]	Swelling power [n]	Solubility [n]	Microscopical appearance
°C	J/g (cal/g)	%	%		%	
—	10.52 ± 0.29 (2.51 ± 0.07) [3]	0† [3]	0.1 ± 0.4† [4]	2.0 [4]	0.2 [4]	Intact birefringent granules
47	9.02 ± 0.17 (2.15 ± 0.04) [4]	14.2‡ [4]	14.1 ± 1.3‡ [4]	2.4 [4]	0.4 ± 0.1 [4]	Increased swelling and decreased number of granules showing birefringence
50	6.64 ± 0.25 (1.59 ± 0.06) [5]	36.9‡ [5]	47.3 ± 1.9‡ [6]	2.8 [3]	0.6 ± 0.1 [3]	
53	3.04 ± 0.03 (0.73 ± 0.01) [3]	71.1‡ [3]	91.9 ± 0.4‡ [4]	3.9 [3]	0.8 ± 0.1 [3]	
56	1.59 ± 0.02 (0.38 ± 0.01) [2]	85.0‡ [2]	97.2 ± 0.7‡ [2]	5.4 ± 0.1 [3]	1.4 ± 0.1 [3]	
59	0.42 (0.10) [2]	96.0‡ [2]	97.8‡ [2]	6.1 ± 0.2 [3]	1.6 [3]	
65	0 [2]	100§ [2]	—	6.9 ± 0.2 [3]	2.8 [3]	Extensively swollen nonbirefringent granules
100	0 [2]	100§ [2]	—	—	35.0 ± 4.0 [3]	Granules disintegrated

* Mean ± SEM.
† Raw.
‡ Partly gelatinized.
§ Completely gelatinized.

physical and morphological characterization of the starch.

Materials and methods

Preparation of starch samples

Raw commercial wheat starch was obtained from KEBO Lab AB (Stockholm, Sweden). The starch content was 98.2 g/100 g (polymer weight, dry basis), determined as glucose after incubating the sample with a thermostable α-amylase at 95 °C and amyloglucosidase at 60 °C (20). To obtain starch with different DGs, 100 g/L suspensions of wheat starch in distilled water were heated under gentle agitation at 47, 50, 53, 56, 59, or 65 °C. After 20 min the samples were cooled to room temperature. A boiled sample was prepared by boiling a 50 g/L suspension for 20 min.

Degree of starch gelatinization

The DG was measured by two different methods. Duplicate analyses were performed on each preparation. With the calorimetric method (differential scanning calorimetry [DSC]) we measured the heat of gelatinization (ΔH) of the starch samples, ie, the energy that has to be supplied to obtain complete starch gelatinization. Ten to 15 mg of a 100 g/L suspension was heated from 22 to 80 °C at a scanning rate of 10 °C/min in coated sample pans. ΔH was calculated from the peak area of the thermogram. The instrument used was a Perkin-Elmer DSC-2 (Perkin-Elmer Corp, Eden Prairie, MN). The DG was calculated by comparing ΔH for raw starch with that for heat-treated starch:

$$DG\ (\%) = (1 - [\Delta H_{heat\text{-}treated}/\Delta H_{raw}]) \times 100 \qquad (1)$$

The enzymatic method is based on the principle that gelatinized starch is easily digested by glucoamylase to form glucose. A small amount of the heat-treated starch suspensions corresponding to 20 mg of starch was withdrawn and analyzed according to Chiang and Johnsson (21). DG was expressed as the percentage of the total starch content that was immediately susceptible to glucoamylase.

Swelling and solubility patterns

The heat-treated suspensions were carefully transferred to weighed centrifuge bottles and diluted with distilled water to 30 g/L starch concentration. A suspension of raw starch was kept for 20 min at room temperature under gentle agitation. The samples were centrifuged for 15 min at 700 × *g*. The supernatant was decanted and evaporated to dryness in the oven and the amount of solute was measured. Granule swelling and solubility were calculated according to Leach et al (22):

Swelling power

$$= \text{weight of gel}/(\text{total starch} - \text{weight of solutes})\ (g/g) \qquad (2)$$

Solubility (%)

$$= 100 \times \text{weight of solutes/total starch}\ (g/g) \qquad (3)$$

Microscopy

A few drops of a 10 g/L suspension were examined by light microscopy and by polarized light microscopy to further characterize the morphology of the granules.

Experiments in vitro

A porcine pancreatic α-amylase preparation (27 mg protein/mL, 1200 units/mg; Sigma Chemical Co, St Louis, MO) was diluted 1:20 or 1:400 before incubation. The heat-treated samples were diluted with distilled water to 50 g/L. Forty-five milliliters of 0.05 mol/L Na,K-phosphate buffer (0.025 mol/L each of KH_2PO_4 and Na_2HPO_4) containing 0.4 g/L NaCl (pH 6.9) and 1.25 mL of diluted α-amylase preparation were added to a 10-mL subsample corresponding to 500 mg starch. Samples were taken before and after 5–60 min incubation (under gentle

agitation at 37 °C) and analyzed with dinitrosalicylic acid for content of reducing sugar (23). A standard curve was prepared using maltose. The extent of hydrolysis (the proportion of starch degraded to maltose, or percent maltose equivalents) was calculated as 100 times milligrams of maltose equivalents times 0.95 divided by milligrams of starch in sample.

Experiments in vivo

The availability of starch for digestion and absorption in vivo in male rats (Sprague-Dawley, 120–150 g) was studied by analyzing concentrations of glucose and insulin in plasma at various time intervals after gastric intubation. The starch samples were diluted with a NaCl solution to 40 g/L. The final concentration of NaCl was 9 g/L. After being starved for 24 h unanesthetized rats were intubated with a starch suspension (2.5 mL) corresponding to 100 mg starch. Nine rats were used for each sample tested. Blood was obtained from the rats by multiple serial sampling from the retroorbital venous plexus. After 135 min the rats were killed by a blow on the head and a piece of the liver (~0.5 g) was taken from the ventral part of the left lateral lobe for analysis of glycogen content. Estimation of plasma glucose concentration was done with a glucose-oxidase method (24). Plasma insulin levels (IRI) were analyzed with a radioimmunoassay (25). Liver glycogen concentration was determined by the method of Rerup and Lundquist (26).

Statistical evaluation

The results are given as mean ± SEM. The significance of differences was tested with Student's *t* test.

Results

The physical and morphological characterization of the starch samples used in the in vitro and in vivo experiments are presented in **Table 1.** With increasing DG there was an increased susceptibility to glucoamylase as well as a gradual loss of birefringence, indicating that the highly ordered structure within the granules was destroyed to different extents. The soluble starch fraction was only slightly increased for the partly gelatinized samples and for the completely gelatinized sample heated at 65 °C (just above the gelatinization temperature range). In contrast, after boiling, a large fraction of the starch was soluble, indicating disintegration of the granular structure.

The susceptibility of starch with different DGs to hydrolysis by porcine pancreatic α-amylase (added at a concentration of 200 units/g starch where 1 unit liberates 1 mg maltose from soluble starch in 3 min at pH 6.9 and at 20 °C) is shown in **Figure 1.** Raw starch was hydrolyzed most slowly and the susceptibility to α-amylase increased with DG. The two completely gelatinized samples (DG = 100%) and starch with a DG of 96% displayed the highest availability; no significant difference was observed between these three samples.

When a large excess of enzyme was used by increasing the enzyme concentration 20-fold to 4050 units/g starch, the initial hydrolysis rate increased as did the extent of hydrolysis (**Fig 2**). However, the differences in availability between the samples, especially in the initial hydrolysis rate, were still very pronounced. The boiled sample was hydrolyzed to 75% (maltose equivalents) within 5 min and reached a plateau at 80% after 30 min, whereas the hydrolysis values at 5 min for raw starch and starch with DGs of 14 and 37% were 12, 30, and 48%, respectively.

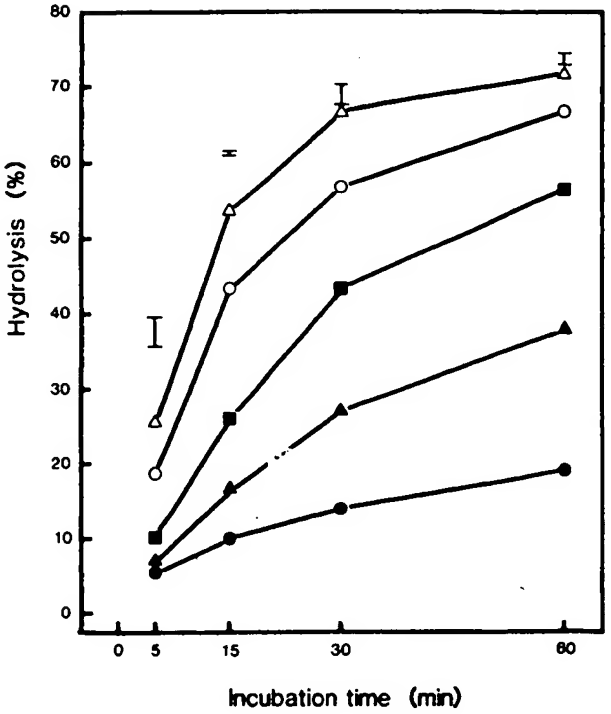

FIG 1. Hydrolysis of starch with different DGs by porcine pancreatic α-amylase added at a concentration of 200 units/g starch. Percentage hydrolysis is expressed in terms of maltose equivalents. DG (%): ●, 0 (raw); ▲, 14.2; ■, 36.9; ○, 71.1; △, 85.0. The bars represent the interval obtained with 96.0, 100 (heated at 65 °C, just above gelatinization temperature range), and 100% (boiled). The SEM did not exceed 3.4 for any experimental point. $n = 2$–3.

The course of the plasma glucose and insulin responses in rats after intubation with starch with different DGs are shown in **Figures 3 and 4** and the area under the curves are presented in **Table 2.** The plasma glucose response after intubation with boiled starch (DG = 100%) was much greater than after raw starch (DG = 0%). The responses to the partly gelatinized products were intermediate. The response to the partly gelatinized starch with a DG of 37% was greater than to that with a DG of 14%. With raw starch the glucose peak was delayed (peak value at 60 min), whereas the peak values for the partly and completely gelatinized starch samples were obtained after only 15 min. The insulin responses were closely associated with the glucose responses. The rise in plasma glucose (0–60 min) and plasma insulin (0–30 min) reflect the early rates of digestion and absorption, where the largest differences are found. The liver glycogen contents 135 min after intubation were 4.1

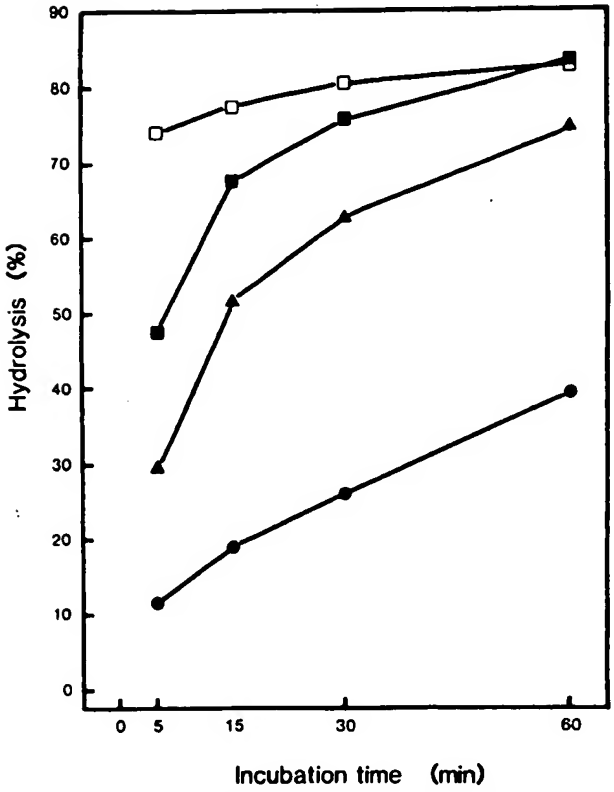

FIG 2. Hydrolysis of starch with different DGs using a large excess of porcine pancreatic α-amylase (4050 units/g starch). DG (%): ●, 0 (raw); ▲, 14.2; ■, 36.9; □, 100 (boiled). The SEM did not exceed 3.4 for any experimental point. n = 2–3.

± 0.7 (raw), 6.9 ± 0.8 (DG = 14%), 6.1 ± 1.1 (DG = 37%), and 6.3 ± 0.5 (boiled) mg/g wet wt of liver. The glycogen content was significantly lower with raw starch than with boiled starch or starch with DG of 14% ($p < 0.05$). The liver glycogen content in control rats (n = 4) intubated with 0.9% NaCl solution was 0.3 ± 0.1.

The plasma glucose and insulin responses were positively correlated with the rate of hydrolysis with α-amylase in vitro, and both the plasma glucose and insulin responses as well as the rate of hydrolysis with α-amylase were positively correlated with the DG (**Table 3**).

Discussion

During gelatinization inter- and intramolecular hydrogen bonds are broken. This results in a loosening up of the compact granular structure and allows different degrees of swelling and absorption of water; fully hydrated starch molecules leach from the granule. Consequently, the availability of the starch granules to digestive enzymes increases to different levels with increasing DG. Even when we used an enzyme concentration in our in vitro system that was so high that the boiled wheat starch was hydrolyzed almost completely within 5 min, large differences in the rate of hydrolysis remained (Fig 2). Starch hydrolysis with α-amylase results in the formation of glucose, maltose, maltotriose, and α limit dextrins (branched oligosaccharides with four glucose monomers or more). Therefore, 75–80% hydrolysis expressed as maltose equivalents corresponds to an almost complete hydrolysis. Once the highly organized structures within the swollen granules were completely destroyed, as in the sample treated just above the gelatinization temperature range, the availability of starch was not increased further by swelling, rupture, and disintegration of the granular structure (boiled sample) (Fig 1). The increased susceptibility to α-amylase was not related to increased solubility because the soluble starch fraction did not exceed 3% in any sample, except for boiled starch.

The plasma glucose and insulin concentrations in rats were raised to different extents depending on the DG. An already low DG (14%), which caused only slight swelling and internal disorganization of the granules (Table 1), raised the glycemic response far above that of raw starch. The decline in plasma glucose concentration was slower for partly gelatinized starch than for completely gelatin-

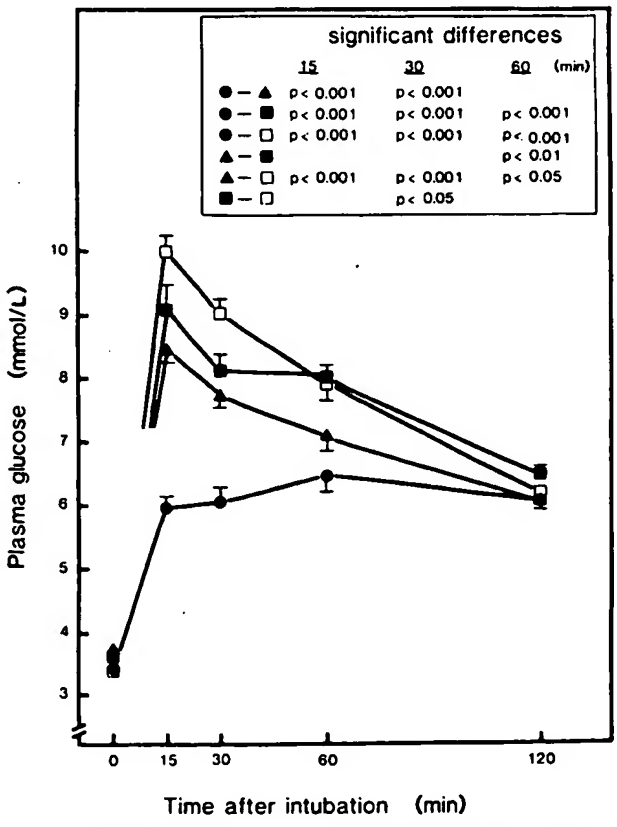

FIG 3. Plasma glucose responses at various intervals in rats given starch with different DGs (100 mg as a 40 g/L suspension in 9 g/L NaCl). DG (%): ●, 0 (raw); ▲, 14.2; ■, 36.9; □, 100 (boiled). Nine rats were used for each sample tested. (Mean ± SEM.)

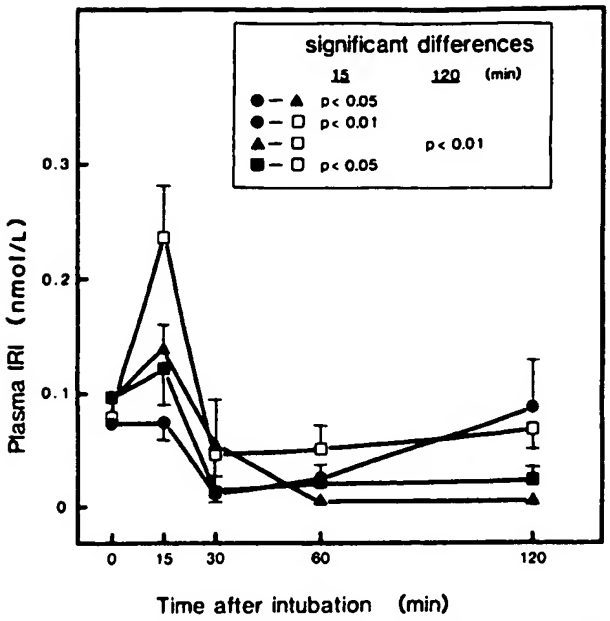

FIG 4. Plasma insulin (IRI) responses at various intervals in rats given starch with different DGs (100 mg as a 40 g/L suspension in 9 g/L NaCl). DG (%): ●, 0 (raw); ▲, 14.2; ■, 36.9; □, 100 (boiled). Nine rats were used for each sample tested. (Mean ± SEM.)

ized starch, which confirms that digestion and absorption are prolonged when swelling is limited. Raw starch was absorbed very slowly and elicited a flattened plasma glucose curve with a delayed and less pronounced peak and a very slow decline.

The effect of complete gelatinization on metabolic response has been studied in humans, both in diabetic and nondiabetic subjects (5–7) and in animals (3, 4). Potatoes and corn starch give much lower postprandial glucose and insulin responses in raw form than after cooking (5–7). In fact, raw corn starch has been used clinically to provide glucose with prolonged absorption in the treatment of type 1 glycogenosis (27). The effect of raw starchy foods on the glycemic response may be more favorable than supplementation with gel-forming types of dietary fiber. The use of raw plant food in the nutrition of diabetic patients was discussed (28).

TABLE 2
Area under the curve after gastric intubation of rats with starch with different DGs*

DG	Area under the curve: Plasma glucose† (0–60 min)	Plasma glucose‡ (0–120 min)	Plasma insulin (0–30 min)
%	*mmol/L × min*	*mmol/L × min*	*nmol/L × min*
0	126 ± 13	282 ± 27	−0.31 ± 0.66
14	211 ± 10 †	381 ± 19 ‡	0.35 ± 0.53
37	§ 248 ± 15 †	§ 465 ± 22 †	−0.25 ± 0.55
100	§ † 293 ± 11 †	† 512 ± 25 †	§ 2.12 ± 0.68 §

* Mean ± SEM; $n = 9$. For each bracket, values without footnotes were significantly different from values with footnotes.
† $p < 0.001$.
‡ $p < 0.01$.
§ $p < 0.05$.

TABLE 3
Correlation coefficients between DG, digestion rate of starch in vitro, and plasma glucose and plasma insulin responses in rats*

	r
DG with digestion rate ($n = 7$)†	0.96
DG with plasma glucose ($n = 4$)	0.88
DG with plasma insulin ($n = 4$)	0.90
Digestion rate with plasma glucose ($n = 4$)	0.98
Digestion rate with plasma insulin ($n = 4$)	0.76

* DG was calculated from calorimetric measurement of the heat of starch gelatinization. Digestion rate = degree of hydrolysis after 60 min incubation with α-amylase (added at a concentration of 200 units/g starch). Plasma glucose = area under the curve, 0–60 min. Plasma insulin = area under the curve, 0–30 min.
† n = number of starch samples with different DGs on which the calculations were based.

Despite a low enzymatic availability, raw wheat starch is almost completely digested and absorbed in the rat small intestine (29). Raw potato starch, on the other hand, is poorly digested (30).

In some processed food products the starch is only partly gelatinized with a slightly swollen granular structure and the internal granular organization partly intact. For example, some baked products and breakfast cereals contain incompletely gelatinized starch granules, which is attributed to the limited water available (10–60 g/100 g) when these products are produced. Cereal flakes are produced by steaming whole kernels and then flaking them between rollers. Starch in breakfast cereals or precooked convenience foods produced under more severe conditions (elevated temperatures, pressures, and shear forces, eg, extrusion cooking or popping) is normally completely gelatinized despite the low water content. A recent study (3) found that the plasma glucose and insulin responses in rats were lower after ingestion of wheat flakes with a DG of 45%, as measured by DSC, than after boiled completely gelatinized whole-grain wheat flour. The in vivo responses were closely related to the in vitro digestibility with pepsin and α-amylase.

According to Wootton and Chaudhry (11) DGs in short bread, hard sweet cookies, soda crackers, crispbread, wafer, fruit cake, and bread crumbs were 1, 2, 3, 33, 40, 50, and 60%, respectively. The corresponding in vitro digestibility values with porcine pancreatic α-amylase were 18, 25, 33, 43, 56, 66, and 69%, respectively whereas pregelatinized wheat starch was digested to 90%. The variations in gelatinization were explained in terms of prebaking water content, baking time at high moisture level favoring high DG, and the presence of added ingredients that restrict gelatinization. Lineback

and Wongsrikasem (10) found DGs between 4 and 97% in several commercial U.S. baked products. Hagander et al (31) observed that extruded crispbread elicited larger glucose and insulin responses than a conventionally baked bread in diabetic patients, possibly because the conventionally baked bread had a lower DG.

Snow and O'Dea (1) obtained a more rapid hydrolysis of starch in vitro in commercial breads compared with homemade breads and suggested that this might have been due to a greater exposure to heat in the commercial breadmaking process. Lüder et al (32) reported significantly lower blood glucose levels in healthy subjects after ingestion of 85% rye–15% wheat bread with a baking time of 35 min compared with bread with a baking time of 45, 55, or 65 min. These differences in glycemic response were probably caused by differences in DG that were caused by the variations in baking time.

The extent of starch gelatinization also varies within a given bakery product because of a moisture gradient (8). Thus, starch gelatinization in white pan bread ranged from 33% in the crust to 70% in the center of the crumb (8). The crust contained many unswollen birefringent starch granules whereas starch granules in the crumb center were deformed and only slightly birefringent. The differences in DG between crumb and crust is the probable reason for differences in postprandial hyperglycemia after ingestion of bread with different crust-to-crumb ratios (33). Bread rolls with high crust-to-crumb ratios raised the blood glucose level more slowly with a later peak and a slower decline than loaves with low crust-to-crumb ratios.

Dreher et al (34) obtained a higher in vitro digestibility and more extensive gelatinization of starch in potato chips compared with starch in baked potato. The in vitro digestibility of potato chips, baked potato, and raw potato were 66.3, 53.6, and 22.8%, respectively.

Differences in the extent of gelatinization after processing legumes in different ways was suggested as a plausible explanation for differences in in vitro starch digestibility (2). It has been suggested that the low metabolic response and in vitro starch digestibility of legumes is caused by the entrapment of starch in the cells (12, 13, 35). The cell walls may limit the hydrolysis rate because of steric hindrance to enzymatic attack (13). Furthermore, the cell walls may also inhibit starch gelatinization by physically limiting the degree of swelling of the starch granules and by limiting the water transport necessary for complete gelatinization (12, 13). Grinding raw legumes, thus disrupting the cell walls, followed by cooking resulted in an increased swelling of the granules, and it was proposed that this could increase the rate of starch digestion as well as the glycemic response (12, 13).

DSC is a calorimetric method based on the physical course of gelatinization but it requires equipment not usually available in most nutritional laboratories. However, the values for DG obtained with the simple and rapid enzymatic method using glucoamylase were closely correlated (r = 0.99) with those obtained with DSC (Table 1).

The characterization of the starch, especially regarding the degree of gelatinization, would be desirable in studies concerned with the rate of digestion and absorption of starchy foods. Light microscopy in combination with polarized light microscopy as well as enzymatic methods like that used in this study are rapid methods that are well suited for this purpose.

In conclusion, the rate of starch hydrolysis in vitro and the glucose and insulin responses in vivo are increased to different extents, depending on DG. Hence, starchy foods with low DGs may be beneficial nutritionally because they favor a reduced rate of starch uptake.

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APPENDIX D

Bioavailability of starch in various wheat-based bread products: evaluation of metabolic responses in healthy subjects and rate and extent of in vitro starch digestion[1-3]

Jörgen Holm and Inger Björck

ABSTRACT Glucose and insulin responses to bread products were evaluated in healthy subjects. Also studied were the rate of in vitro starch digestion and the content of in vitro resistant starch (RS). Three white-wheat-bread (WWB) products varying in crust-crumb ratio and monoglyceride addition, three bread products with a high soluble fiber content (HSFB), and two coarse-wheat breads (CB) were included. The metabolic responses to WWBs were in general higher than those to CB and HSFB products. The most prominent reduction in metabolic responses was noted with the CBs with intact kernels and the HSFBs with oat bran. The starch in these products was also more slowly released from a dialysis tubing after enzyme incubation of chewed samples. The RS content ranged from 0 to 1.7 g/100 g starch. HSFBs and the CB with intact kernels showed a higher satiety score than did the WWBs immediately after the test meal. *Am J Clin Nutr* 1992;55:420–9.

KEY WORDS White bread, whole grains, soluble fiber, resistant starch, starch digestion rate, glycemic and insulinemic responses, healthy subjects, satiety

Introduction

The glycemic responses to starchy foods vary greatly depending on factors such as the botanical origin or the type and extent of food processing (1–8). This is of consequence in the dietary management of important metabolic disorders, including diabetes (1, 2) and hyperlipidemia (9). The glycemic response and consequently the insulin demand appear to be closely related to the enzymic susceptibility of starch (10) as well as to the rate of gastric emptying (6). Much work has been done to evaluate mechanisms behind differences in the rate of digestion and absorption (3, 5–7, 10) and to rank starchy foods by their effect on the glycemic response (1, 2, 4–8). In many of these studies conventional white bread has been identified as a rapidly digested and absorbed food that elicits high glucose and insulin responses (1, 6, 8, 10–14). This is probably related both to a rapid gastric emptying (6) and to a high rate of starch digestion (3).

The high metabolic responses to conventional breads are unfortunate. Bread makes up a considerable part of our intake of both starch and dietary fiber. It constitutes the main part of one or more of the daily meals and cannot easily be replaced in contrast to other rapidly digested base foods, eg, potato. Most results reported concern white wheat bread. Studies with wholemeal wheat bread indicate no effects of dietary fiber in wheat per se (4). However, a more intact botanical structure as in bulgur wheat (parboiled cracked grains) (14) as well as the firm physical structure of pasta products (15) produce lower glycemic responses than does a corresponding wheat bread made from milled flour. No data are available concerning the effect of incorporating intact wheat kernels in bread. However, the glycemic response to bread appears to be lower after incorporation of whole or cracked rye (4, 12) or barley (14) grains. The metabolic response to bread may also be reduced by the addition of viscous types of dietary fiber, such as guar gum (16), or by replacement of wheat flour with flour types richer in viscous dietary fiber components, eg, rye flour (17).

Another interesting nutritional variable with respect to starch in bread is the formation of in vitro resistant starch (RS). RS, analytically defined as starch resistant to amylases in vitro unless solubilized in alkali or dimethylsulfoxide (18, 19), is formed during baking (18–21) as well as during several other types of heat treatment (21–23) and has been shown to consist of retrograded amylose (24, 25). Nutritionally, RS in cereal products appears to resemble certain types of soluble dietary fiber in that it passes undigested through the small intestine (20, 21, 23) but is easily fermented in the hind gut (20, 23). To a certain extent delivery of starch to the colon microflora seems to be desirable (26). The amount of RS delivered daily to the large bowel by a Western diet was recently estimated at 2.5–5 g (8), which is considerable. In view of the quantitative importance of bread in our diet, bread can be expected to be one of the major sources of RS. However, the actual amount in a bread product may vary because of variations in formulas and baking conditions (18, 19).

The purpose of the present study was to evaluate means of improving the nutritional properties of starch in bread. Attempts were also made to explore possible mechanisms for the differences in metabolic responses to white wheat bread reported in the literature. The effects on glycemic and insulinemic responses as well as the satiety scores for various bread products were evaluated in healthy subjects. In parallel to the clinical studies, starch

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digestion rate was measured in a recently developed in vitro system employing enzyme incubation of chewed rather than artificially disintegrated products in dialysis tubings. The RS content in the various bread products was also determined.

Materials and methods

Bread products

White wheat flour, whole-grain wheat, and oat bran were obtained from Nord Mills AB (Malmö, Sweden). The commercial oat bran is prepared by steaming dehusked whole oat grains followed by milling and bolting and contains (wt/dry wt basis) 21% dietary fiber (13% insoluble and 8% soluble), 47% starch, 20% protein, and 8% fat. The spaghetti used contained exclusively durum wheat with added monoglycerides (Storhushålls spagetti, Kungsörnen AB, Järna, Sweden). Linseeds (AB R Lundberg, Malmö, Sweden) contained (dry weight basis) 43.7% dietary fiber (36.3% insoluble and 7.4% soluble), 24% protein, and 31% fat.

Three variants of white wheat bread (WWB) were baked: loafs with added monoglycerides (WWB-mg) and without added monoglycerides (WWB-tl) and rolls without added monoglycerides (WWB-r). All WWBs were baked from 3700 g white wheat flour, 200 g baker's yeast, and 2000 g water. In the case of WWB-mg, 37 g monoglycerides were added. The doughs were proofed for 20 min at 28 °C. Subsequently, they were divided into 440-g pieces and put in aluminium pouches or divided into 60-g pieces (WWB-r) and formed as rolls followed by a second proofing for 35 min (38 °C, 75% relative humidity). Baking was performed at 210 °C (200 °C for rolls) for 22 min (20 min for rolls) in a convection oven with steam added during the first 30 s.

Two variants of coarse bread (CB) were baked: whole-grain wheat bread composed of intact wheat kernels (80%) and white wheat flour (20%) (CB-wwg) and an experimental bread composed of 70% spaghetti cuts and 30% white wheat flour (CB-sp). CB-wwg was baked according to the following recipe: 2960 g whole-wheat grains were boiled in 2960 g water for 20 min and then cooled. To this 740 g white wheat flour, 200 g yeast, and 1200 g water were added. Proofing and baking were performed as with WWB-tl except that the first proofing was increased to 30 min. The doughs were divided into pieces of 580 g and baking time was increased to 45 min at 200 °C. CB-sp was baked according to the following recipe: 2600 g cuts of spaghetti (1–1.5 cm) were soaked in 2000 g water for 30 min. Subsequently, 1100 g white wheat flour and 200 g yeast were added. Proofing and baking were performed as with WWB-tl except that the first proofing was extended to 30 min. The doughs were divided into pieces of 550 g and baking time was increased to 25 min.

Three variants of high-fiber breads rich in soluble dietary fiber (HSFB) were baked: oat bran bread (HSFB-ob), linseed bread (HSFB-ls), and one product baked from a commercial blend that contained a mixture of high-fiber ingredients (HSFB-mf). HSFB-ob was baked according to the following recipe: 1665 g oat bran, 40 g yeast, and 3300 g water were mixed and the dough was left to rest for 16 h. To this dough 1850 g white wheat flour, 185 g wheat gluten, 170 g yeast, and 300 g water were added. Proofing and baking were performed as with WWB-tl except that doughs were divided into pieces of 550 g and baking time was increased to 25 min. HSFB-ls was baked according to the following recipe: 500 g linseed in 3000 g water was brought to boil to extract water-soluble-fiber components and the decoction was strained off. In another batch 925 g linseed in 1500 g water was brought to boil and cooled. To this batch 1500 g of the decoction of linseed, 2775 g white wheat flour, and 200 g yeast were added. Proofing and baking were performed as with WWB-tl except that baking time was extended to 30 min.

The formula for HSFB-mf consisted of 3450 g of a commercial mixture containing white wheat flour and various fiber sources (Fiberform, Nord Mills AB), 100 g yeast, and 2000 g water. In addition to flour and fiber ingredients the mixture contained several types of baking improvers, ie, amylase, ascorbic acid, and cysteine. The ingredients in the mixture are shown in **Table 1**. The baking time was extended to 27 min; otherwise the procedure was the same as with WWB-tl.

Fifty grams NaCl and 50 g sucrose (as yeast substrate) were included in all recipes. After cooling each bread was put in a plastic bag and stored at room temperature for 16 h. Subsequently, the bread was cut into slices (ends were discarded), except for rolls, and put in aluminium foil and plastic bags and stored at −20 °C until used.

Chemical analysis and physical characterization

The products were dried in a vacuum oven and milled (< 0.8 mm; Cyclotec, Tecator, Sweden) before analysis. The bread products were analyzed for contents of starch (27), total and soluble dietary fiber (28), and β-glucans (only HSFB-ob) (29). Aqueous suspensions of samples were examined by polarized light microscopy to characterize the starch granules. The composition of the bread products is shown in Table 1.

WWB products were also analyzed for volume (measured by seed replacement) and proportion of crust. The blue value (BV), which is proportional to the amount of uncomplexed (lipid-free) amylose present in starch, was determined (30) and used to estimate the fraction of amylose complexed with lipids. The fraction of complexed amylose in the product was calculated as BV for lipid-free starch minus BV for product divided by BV for lipid-free starch. Pure commercial potato starch (Stärkelsen, Kristianstad, Sweden), which is free of lipids and with an amylose content close to that of wheat starch, was used as lipid-free starch reference.

The proportion of crust amounted to 37 ± 3% (wt:wt, dry basis, $n = 3$) in WWB-r, which was higher than the proportion in the corresponding white loafs (WWB-mg and WWB-tl) (24 ± 2%, $n = 3$). Addition of monoglycerides increased the bread volume of white loafs by 10%. The specific volumes were 3.4 and 3.8 cm^3/g for WWB-tl and WWB-mg, respectively. Further, the crumb was much softer and more porous after addition of monoglycerides. The fraction of amylose complexed with lipids was only slightly higher in WWB-mg (50.0 ± 1.4%) than in the two other WWBs (46.0 ± 1.2) ($n = 5$). No striking differences in the appearance of starch granules were observed when the different bread products were examined with polarization microscope (data not shown).

Rate of in vitro starch digestion

The digestion rate of milled samples was measured by incubating the samples with porcine pancreatic α-amylase (Sigma Chemical, St Louis) with and without a prior 60-min incubation with pepsin at pH 1.5 (31). The α-amylase concentration was 200 U/g starch (1.8 U/mL sample suspension; 1U liberates 1 mg maltose from soluble starch in 3 min at pH 6.9 and 20 °C). Samples were withdrawn before and after 15- and 120-min incubations for determination of reducing sugar content. The ex-

TABLE 1
Ingredients and composition of the breads based on dry and wet (values in brackets) weights*

Product	Ingredients	Starch	Dietary fiber†		Protein	Fat	Energy	Water
			Total	Soluble				
		g/100 g	g/100 g	g/100 g	g/100 g	g/100 g	kJ	g/100 g
White wheat breads								
WWB-mg	White wheat flour: monoglycerides, 99:1	79.7 [49.7]	3.5 [2.2]	1.1 [0.7]	10.6 [6.6]	2.2 [1.4]	1637 [1022]	— [38]
WWB-tl	White wheat flour	80.3 [49.7]	3.7 [2.3]	1.1 [0.7]	10.7 [6.6]	1.1 [0.7]	1604 [992]	— [38]
WWB-r	White wheat flour	79.9 [56.7]	3.7 [2.6]	1.1 [0.8]	10.6 [7.5]	1.1 [0.8]	1595 [1135]	— [29]
Coarse breads								
CB-wwg	White wheat flour: whole wheat grains, 20:80	69.6 [34.4]	11.4 [5.6]	1.2 [0.6]	11.7 [5.8]	2.4 [1.2]	1491 [737]	— [51]
CB-sp	White wheat flour: spaghetti cuts, 30:70	76.0 [47.1]	4.0 [2.5]	1.2 [0.7]	12.8 [7.9]	1.1 [0.7]	1566 [971]	— [38]
High-soluble-fiber breads								
HSFB-ob	White wheat flour: oat bran: gluten, 50:45:5	61.4 [31.8]	10.9 [5.7]‡	4.6 [2.4]	19.7 [10.2]	4.2 [2.2]	1558 [808]	— [48]
HSFB-ls	White wheat flour: linseed, 75:25	58.8 [33.6]	13.5 [7.7]	3.9 [2.2]	15.1 [8.6]	10.0 [5.7]	1658 [946]	— [43]
HSFB-mf	White wheat flour, linseed, beet fiber, soybean pieces, maize fiber, pea fiber, sunflower seeds, gluten, oil, lecithin, cysteine, amylase, ascorbic acid	58.8 [37.4]	15.1 [9.6]	3.4 [2.2]	14.2 [9.0]	7.1 [4.5]	1528 [971]	— [36]

* Values for protein, fat, and energy are calculated from food tables. All other values are based on analyses of the breads.
† Dietary fiber figures corrected for resistant starch.
‡ β-glucans made up 42% of the total dietary fiber content.

tent of α-amylolysis was calculated as the proportion (%) of starch degraded to maltose. A digestion-rate index was expressed as the extent of hydrolysis after 120 min with respect to the extent of hydrolysis with WWG-mg after 120 min.

The digestion rate was also measured by using chewed products incubated with pepsin and subsequently with added α-amylase in dialysis tubing, by a recently developed procedure (15). Six subjects participated in the study. They were told not to eat within 1 h of the experiment. Samples from the central part of the crumb were cut off and used immediately. All portions contained 1 g starch. The samples were given in a randomized order on consecutive days. The subjects were told to rinse their mouths with tap water and subsequently chew the product 15 times (30 times in some experiments). The products were then expectorated into a beaker containing 50 mg pepsin (2000 FIP-U/g, Merck, Darmstadt, FRG) in 6 mL 0.05 mol Na,K-phosphate buffer/L (containing 0.4 g NaCl/L) adjusted to pH 1.5 with 2 mol HCl/L. Finally, the subjects rinsed their mouths with 5 mL phosphate buffer (pH 6.9) for 60 s and the rinsing solution was also transferred into the beaker. The contents were stirred and pH was adjusted to 1.5 with 2 mol HCl/L. Each sample was incubated at 37 °C for 30 min with mixing every 10th min. After the peptic digestion the pH was readjusted to 6.9 with 2 mol NaOH/L, and 1 mL diluted porcine pancreatic α-amylase (Sigma Chemical) suspension containing 110 U was added. The mixture was then diluted to 30 mL with phosphate buffer and transferred to a dialysis tubing (13-cm strips Spectrapor 2, width 45 mm). The bag was turned around 10 times to mix the ingredients and then placed into a beaker containing 800 mL phosphate buffer at 37 °C and gently agitated. Samples (2 ml) of dialysate were taken every 30 min up to 3 h and were analyzed for reducing-sugar content. A standard curve was prepared by using maltose. The extent of hydrolysis was calculated as 100 × mg maltose equivalents × 0.95 ÷ mg starch in sample. An hydrolysis index was calculated as 100 times the sum of hydrolysis values (30–180 min) for the product divided by the sum of hydrolysis values for the WWB-mg product chewed by the same individual.

Resistant starch

RS, ie, starch that is resistant to amylases in vitro unless solubilized in KOH, was determined as previously described (20). An amount of the dietary fiber residue obtained from the enzymatic gravimetric fiber analysis (28) was incubated with amyloglucosidase with and without prior solubilization in 2 mol KOH/L for 30 min. Liberated glucose was quantified and starch content was calculated as 0.9 × monomer weight. RS was expressed as the amount of starch available to amyloglucosidase after solubilization in KOH minus the amount of starch available without prior KOH treatment.

Glucose and insulin responses in healthy subjects

A group of three men and seven women ate their test meals in the morning after a 12-h overnight fast in random order at intervals of 4–8 d. They were asked to eat the breakfast over a 12–15-min period. The age and body mass index (in kg/m^2) were in the ranges 34–44 y and 18.5–23.5 for men and 26–49 y and 17.5–23.0 for women, respectively. Finger-prick capillary blood samples were obtained before the meal and at 30, 45, 70, 95, 120, and 180 min after the start of the meal for analysis of glucose and at 30, 45, 95, and 120 min for analysis of insulin. Blood glucose concentration was determined with a glucose

TABLE 2
Composition of the breakfast test meals

	Bread	Cheese	Margarine	Coffee or tea*	Starch	Dietary fiber		Protein	Fat	Energy
						Total	Soluble			
	g	g	g	g	g	g	g	g	g	kJ
WWB-mg	101	32	6	220	50	2.2	0.7	15.9	15.5	1733
WWB-tl	101	32	7	220	50	2.3	0.7	15.9	15.6	1738
WWB-r	88	32	7	235	50	2.3	0.7	15.9	15.6	1738
CB-wwg	145	26	8	185	50†	8.1	0.9	15.9	15.6	1738
CB-sp	106	26	9	220	50‡	2.6	0.7	15.9	15.4	1729
HSFB-ob	157	—	15	185	50	9.1	3.9	16.0	15.5	1738
HSFB-ls	149	10	4	195	50	11.5	3.3	15.7	14.6	1696
HSFB-mf	134	14	7	210	50	12.9	3.0	16.1	15.7	1746

* Various amounts were given because of differences in water content of the breads. The total water content was 260 g for all breakfast meals.
† 39 g originates from whole-wheat grains.
‡ 35 g originates from spaghetti cuts.

oxidase peroxidase reagent and plasma insulin concentration with an enzyme immunoassay kit (Boehringer Mannheim, Mannheim, FRG). The glycemic index (GI) was calculated from the 2-h incremental glucose area using WWB-mg as the reference food (GI = 100) (13). WWB-mg was chosen because monoglycerides or lipids are added to most commercially baked white-wheat-bread products. Also, all test products contained either naturally occurring lipids (HSFB-ob, HSFB-ls), added lipids (HSFB-mf), or added monoglycerides (CB-sp; monoglycerides were present in the spaghetti). The insulinemic index was calculated similarly from the insulin response curves (11) with WWB-mg as reference. Approval of the study was given by the ethics committee of the University of Lund, Sweden.

Test meals

The composition of the test meals is shown in **Table 2.** All meals contained 50 g starch (three to four slices of bread). The bread products were served with different amounts of cheese, margarine, and coffee and tea to balance the protein, fat, and water contents of the test meals.

Acceptability and satiety scores

Immediately after finishing the breakfast, the subjects were asked to assess the acceptability of the bread products on a bipolar hedonic scale (32) where −4 represents dislike extremely, 0 represents a neutral response neither like or dislike, and +4 represents like extremely. The satiety score of the test meals was estimated numerically according to Haber et al (33). Assessments were done before the meal and at 15 (immediately after finishing the meal), 95, and 180 min after the start of the meal on a scoring system graded from −10, to represent extreme hunger, to +10, to represent extreme satiety.

Statistical evaluation

Results are given as means ± SE if not otherwise stated. Results were subjected to analysis of variance (bread product, type of bread, and subject were chosen as factors) and multiple comparisons were made by using least significant difference (LSD). The Wilcoxon signed-ranks test was also used to test significance of differences between the white wheat bread with added monoglycerides (WWB-mg) and each other product. Statistical evaluations were performed with the *SPSS/PC+* program (SPSS Inc, Chicago).

Results

All breads except WWB-r and CB-sp received positive acceptability scores (**Table 3**). HSFB-ob received positive ratings from all subjects and showed the highest average score. CB-wwg was liked very much by some subjects and disliked moderately by others. Addition of monoglycerides tended to increase the acceptability of WWB loaves by increasing the softness of the crumb.

All HSFBs and CB-wwg showed a higher satiating effect compared with WWB-mg immediately after the meal was finished (Table 3). However, the satiety scores for HSFBs and CB-wwg

TABLE 3
Acceptability and satiety scores for the different bread products*

Product	Acceptability score†	Satiety score‡		
		15 min	95 min	180 min
WWB-mg	1.3 ± 0.4^{ad}	3.6 ± 0.4^{a}	0.9 ± 0.9	−3.8 ± 0.7
WWB-tl	0.6 ± 0.5^{a}	3.7 ± 0.5^{a}	1.2 ± 0.8	−3.6 ± 0.6
WWB-r	−0.9 ± 0.5§^{b}	4.0 ± 0.3^{ac}	1.1 ± 0.8	−3.6 ± 1.1
CB-wwg	0.8 ± 0.7^{ac}	5.2 ± 0.4§^{b}	2.1 ± 0.7	−3.0 ± 0.8§
CB-sp	−0.3 ± 0.6§^{b}	4.0 ± 0.5^{ac}	1.0 ± 0.8	−3.4 ± 1.1
HSFB-ob	2.5 ± 0.2§^{d}	4.5 ± 0.5§^{abc}	1.8 ± 0.8	−2.3 ± 0.9
HSFB-ls	1.5 ± 0.3^{ad}	5.0 ± 0.4§^{bc}	2.2 ± 0.6	−3.4 ± 0.6
HSFB-mf	1.7 ± 0.4^{ad}	5.1 ± 0.2§^{bc}	2.3 ± 0.6§	−2.6 ± 0.7

* $\bar{x}$ ± SE.
† Means not sharing the same superscript letter are significantly different according to analysis of variance (LSD): $P < 0.01$ except for WWB-r vs WWB-tl, WWB-r vs CB-wwg, WWB-mg vs CB-sp and HSFB-ob vs CB-wwg ($P < 0.05$).
‡ Means not sharing the same superscript letter are significantly different according to analysis of variance (LSD): $P < 0.05$ except for WWB-mg vs CB-wwg ($P < 0.01$). No significant differences were observed at 95 and 180 min.
§ Significantly different from WWB-mg reference $P < 0.05$ (Wilcoxon's signed-rank test).

did not remain significantly above the WWBs at 95 and 180 min with a few exceptions.

The rates of starch hydrolysis with milled samples on incubation with pepsin and α-amylase were similar for the eight bread products. After 15 and 120 min α-amylolysis the degrees of hydrolysis were 39–43% and 71–75%, respectively. Consequently, the hydrolysis indexes for milled samples were close to 100 for all products (**Table 4**). When the peptic-digestion step was omitted, the initial degree of hydrolysis (15 min) decreased by the same magnitude with all samples, on average from 41 ± 2% to 35 ± 2% for the eight bread products, which is equivalent to a decrease of 15%. After 120 min the corresponding figures were 73 ± 2% and 71 ± 2%, respectively.

The digestion rate for chewed products subjected to α-amylolysis in dialysis bags was highest with the two WWBs tested (WWB-mg and WWB-tl) (**Fig 1, Table 4**). The CB and HSFB products used in the experiment (CB-wwg and HSFB-ob) showed significantly lower digestion rates. No changes in the degrees of hydrolysis were observed with WWB-mg and CB-wwg when chewing was extended from 15 to 30 times. After 30-min pepsin incubation, the WWBs were completely disintegrated. A small difference was observed between the two white loaves in that WWB-mg disintegrated more rapidly and a very fine milky suspension was obtained. Microscopic examination showed a suspension of free swollen starch granules. In contrast peptic incubation of chewed CB-wwg resulted in a mixture with intact or partially disintegrated kernels.

RS was detected in all bread products except in HSFB-mf (**Table 5**). Only traces of RS (< 0.1% dry weight basis) were detected in fiber residues from the raw materials. The content of RS was highest in CB-wwg. Of the WWBs, addition of monoglycerides (WWB-mg) or baking as rolls (WWB-r) tended to decrease the RS content compared with that observed in WWB-tl.

The blood glucose responses to the three WWBs did not differ significantly at any point (**Fig 2**, top). However, the initial response (30 and 45 min) tended to be lower after WWB-tl than after WWB-mg but the differences were not significant. In the initial phase both CBs showed significantly lower glucose values than did the WWB-mg reference (Fig 2, middle). CB-sp showed a very slow decline, which resulted in a significantly higher late glucose concentration (180 min) compared with WWB-mg. The initial glucose responses after HSFB-ob and HSFB-mf were significantly lower than after WWB-mg whereas HSFB-ls showed a response similar to that obtained with WWB-mg at all times (Fig 2, bottom).

Apart from the response to HSFB-ls, the insulin responses were closely associated with the glucose responses (**Fig 3**). The insulin response tended to be lower after WWB-tl than after WWB-mg (Fig 3, top) and the difference was statistically significant after 45 min ($P < 0.05$ by Wilcoxon test). The response to CB-wwg was significantly lower at all times except at 30 min (Fig 3, middle). All HSFBs showed significantly lower insulin concentrations than did WWB-mg after 45 and 95 min (Fig 3, bottom).

The GI was significantly lower after CB-wwg than after WWB-mg ($P < 0.05$ by LSD and Wilcoxon tests) (Table 4). The GIs of HSFB-ob and HSFB-mf were also lower than that of WWB-mg according to the Wilcoxon test ($P < 0.05$). The GI group mean for CBs was lower than that of WWBs ($P < 0.05$ by LSD). The insulinemic indexes of CB-wwg and HSFB-ob were significantly lower than those of WWB-mg and WWB-r ($P < 0.05$ for all four differences by both LSD and Wilcoxon tests). The insulinemic indexes of CB-sp and HSFB-ls were also lower than that of WWB-mg according to the Wilcoxon test. The insulinemic index group means for CBs and HSFBs were significantly lower than that of WWBs ($P < 0.01$ and $P < 0.05$, respectively, by LSD). A statistical evaluation based on the areas under the

TABLE 4
In vitro starch digestion rate, area under glucose- and insulin graph, and glycemic and insulinemic indexes of the bread products

Product	In vitro hydrolysis index		Metabolic variables			
	Milled*	Chewed†§	Glucose area	Insulin area	Glycemic index‡	Insulinemic index‡
	%	%	$mmol \cdot L^{-1} \cdot min^{-1}$	$nmol \cdot L^{-1} \cdot min^{-1}$	%	%
WWB-mg	100	100[a]	122.2 ± 14.8§	20.0 ± 2.8	100[a]	100[a]
WWB-tl	102	96 ± 4[a]	98.7 ± 18.6	16.6 ± 2.8	84 ± 12[ab]	88 ± 9[ab]
WWB-r	104	nd	112.7 ± 18.7	19.0 ± 2.9	96 ± 11[ab]	103 ± 12[a]
CB-wwg	105	79 ± 4‖[b]	80.4 ± 12.0‖	14.3 ± 3.8‖	68 ± 9‖[b] ¶	69 ± 8‖[b] ¶
CB-sp	102	nd	93.2 ± 13.3‖	15.6 ± 2.6‖	79 ± 9[ab]	78 ± 6‖[ab]
HSFB-ob	103	86 ± 1‖[b]	89.8 ± 13.6‖	12.9 ± 2.7‖	75 ± 9‖[ab]	67 ± 9‖[b]
HSFB-ls	103	nd	116.6 ± 24.5	14.7 ± 2.5‖	93 ± 15[ab]	77 ± 8‖[ab] **
HSFB-mf	100	nd	90.3 ± 12.6‖	15.5 ± 2.4	78 ± 7‖[ab]	89 ± 15[ab]

* Milled (< 0.8 mm) before peptic-amylolytic digestion in a beaker. Mean of two experiments. The coefficient of variation did not exceed 2.3% for any product.

† Chewed before peptic-amylolytic digestion in a dialysis sac. Mean of six experiments. Means not sharing the same superscript letter are significantly different by analysis of variance (LSD): $P < 0.001$ except for WWB-mg vs HSFB-ob ($P < 0.01$) and WWB-tl vs HSFB-ob ($P < 0.05$).

‡ Means not sharing the same superscript letter are significantly different according to analysis of variance (LSD): $P < 0.05$.

§ $\bar{x} \pm$ SE.

‖ Significantly different from WWB-mg reference by Wilcoxon's signed-rank test: $P < 0.05$.

¶ Group mean for CBs significantly different from group mean for WWBs by analysis of variance (LSD): $P < 0.05$ (glycemic index), $P < 0.01$ (insulinemic index).

** Group mean for HSFBs significantly different from group mean for WWBs by analysis of variance (LSD): $P < 0.05$.

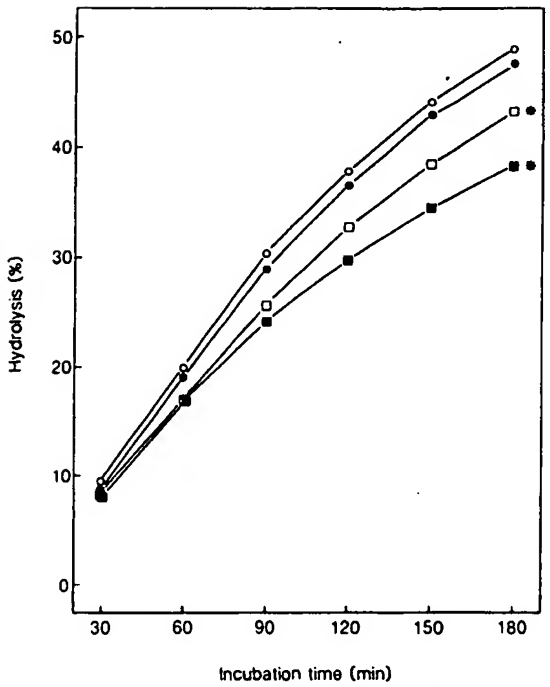

FIG 1. Starch hydrolysis rates with white, high-soluble fiber and coarse bread products that were chewed and incubated with pepsin followed by digestion with α-amylase in dialysis bags: ○, WWB-mg; ●, WWB-tl; □, HSFB-ob; ■, CB-wwg. *Significantly different from WWB-mg at 180 min by Wilcoxon test ($P < 0.05$).

glucose and insulin graphs, respectively, revealed no differences compared with the corresponding evaluation based on GIs and insulinemic indexes (Table 4).

The GIs and insulinemic indexes were closely correlated ($r = 0.78$, $P = 0.02$, $n = 8$). The GIs and insulinemic indexes were also closely correlated with the hydrolysis index using the chewing-dialysis method ($r = 0.95$, $P = 0.05$ and $r = 0.93$, $P = 0.07$, respectively, $n = 4$).

Discussion

The starch hydrolysis rates during incubation of milled preparations with α-amylase were similar with all bread products. Thus, there were no substantial differences related to properties of the starch moiety as such, ie, degree of starch gelatinization (7), starch retrogradation (34), or extent of amylose-lipid complexation (35). Further, the presence of dietary fiber did not affect enzymic action.

The in vitro digestion rates in products that were chewed, incubated with pepsin, and incubated subsequently with α-amylase in dialysis tubings did, however, reveal significant differences. In this case the importance of the physical form will be reflected because the products are studied in the form in which they are usually eaten. In addition possible diffusion resistance by viscous dietary fiber is also considered when employing dialysis. Consequently, when the chewing-dialysis method was adopted, the whole-grain product (CB-wwg) as well as the HSFB tested (HSFB-ob) separated significantly from the WWBs. The chewing-dialysis method seems to offer a possibility to evaluate the importance of food structure and is more physiological than methods using milled, mortared, or whole products. The method has shown good correlation to in vivo data from rice, pasta, and leguminous products (Y Granfeldt, I Björck, A Drews, J Tovar, unpublished observation, 1991).

Protein may physically encapsulate starch, preventing the enzyme access (7, 31). Starch-protein interactions in wheat bread were reported to reduce the glycemic response as well as the total digestibility of starch (36). In the present study the initial rate of α-amylolysis with milled bread products was decreased by ~15% for all products if pepsin was excluded from the in vitro assay. Consequently, this figure was not influenced by crust-crumb relationship or if white flour was replaced by whole grains or oat bran flour. In previous studies in rats we (7, 31) obtained an improved correlation between in vitro data and the glycemic responses to milled wheat products when pepsin was included in the in vitro assay. This indicated that these protein structures did not reduce the glycemic response.

In ordinary bread gluten forms a continuous network that constitutes the backbone. On incubation of the chewed WWB products with pepsin, this network was degraded and the breads disintegrated, resulting in a fine suspension of swollen starch granules highly susceptible to α-amylase. Hence, we have no indications of protein-starch interactions of importance for the metabolic response to either milled cereal products or chewed WWBs.

The rapid disintegration during peptic digestion of WWBs favors a high availability to subsequent amylolysis. It may also explain the rapid rate of gastric emptying reported for white wheat bread (6) because soluble particles empty much more rapidly from the stomach than do solids (37). In contrast to WWBs the CB product with intact kernels was not disintegrated even after a 30-min pepsin incubation of the chewed product. Chewed spaghetti and grains of parboiled rice, both characterized as lente (15, 38), behaved similarly to the CB-wwg product (Y Granfeldt, I Björck, A Drews, J Tovar, unpublished observation, 1991), which supports that a low extent of disintegration in the stomach reduces the metabolic response.

The contents of RS detected in the bread products (< 1.7 g/100 g starch) as well as previously published figures for conventional bread types (0.6–1.0 g/100 g starch) (18–21) are too small to be significant for the glycemic response. However, it may constitute a considerable part of the total amount of malabsorbed starch in humans. Consumption of five to six slices of bread per

TABLE 5
Content of RS in the bread products*

	Dry matter basis	Starch basis
	g/100 g	
WWB-mg	0.6	0.7
WWB-tl	0.8	1.0
WWB-r	0.6	0.7
CB-wwg	1.2	1.7
CB-sp	0.6	0.8
HSFB-ob	0.6	1.0
HSFB-ls	0.3	0.5
HSFB-mf	0	0

* Resistant starch is starch resistant to amylases in vitro unless solubilized in KOH.

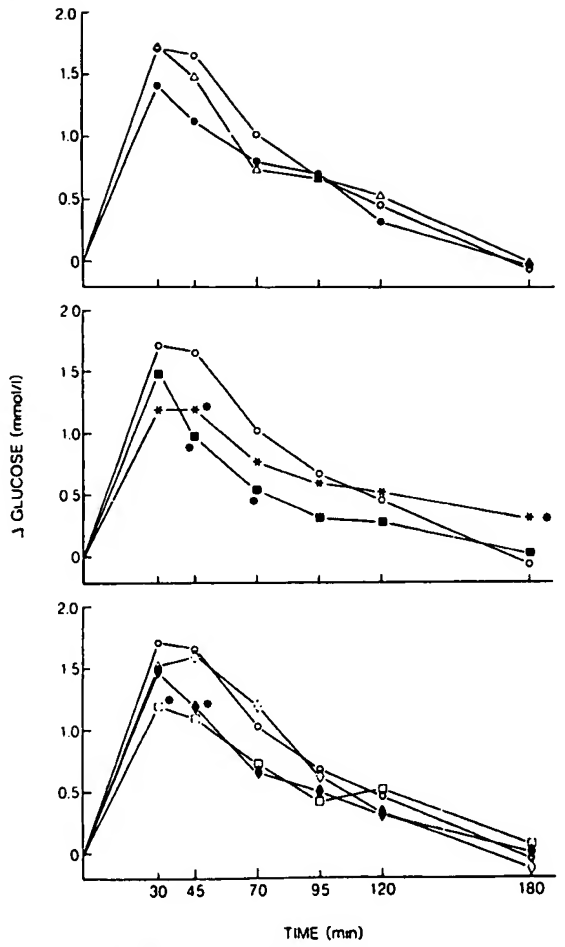

FIG 2. Glucose responses in healthy subjects to breakfast meals with white (top), coarse (middle), and high-soluble-fiber (bottom) breads. Each meal contained 50 g starch. WWB-mg is included as a rapid reference bread in all panels: O, WWB-mg; ●, WWB-tl; △, WWB-r; ■, CB-wwg; •, CB-sp; ◇, HSFB-ls; □, HSFB-ob; ◆, HSFB-mf. *Significantly different from WWB-mg by Wilcoxon test: $P < 0.05$ except for the comparison with CB-sp at 180 min ($P < 0.01$).

day (250–300 g bread) corresponds to an intake of ~2.5–3 g RS. This should be considered in relation to the total amount of malabsorbed starch, which has been estimated to 5–10 g/d (8) and to the daily intake of dietary fiber, which is ~15 g. Several mechanisms may be responsible for malabsorption of starch in food products (21). However, the starch malabsorbed from wheat products seems to be composed mainly of RS (20, 21, 23). Malabsorption of starch implies numerous positive nutritional effects, some of which are mediated through the metabolically active volatile fatty acids produced during fermentation in the large bowel (26).

The contents of RS in the present bread products differed greatly (0–1.7 g/100 g starch). The high RS content in CB-wwg is probably related to the repeated heating and cooling of the starch in the whole grains (boiling to soften the grains and baking) as well as to the higher moisture content in the dough. Both factors were reported to increase RS formation in food products (18, 39). HSFB-mf contained no RS. This was probably due to the presence of amylase in the bread mix, which results in a decreased chain-length distribution of amylose that may be less favorable for RS formation. It is also possible that the dextrins formed may interfere with the retrogradation process. Similarly, no RS was detected in bread baked from malted wheat flour with a high intrinsic amylase activity (40). Obviously, the RS content may be affected by recipe and baking conditions and the present data provide useful information about the RS content of bread products.

As a group the WWBs resulted in a higher GI and insulinemic index compared with the group means of CBs and HSFBs, respectively. There were also slight differences within the WWB group. The WWB-tl tended to elicit a somewhat lower response. However, the differences only were significant for the early insulin response. The two white loaves differed only in that 1% of monoglycerides was included in WWB-mg. The lipid addition only slightly increased the fraction of amylose complexed with

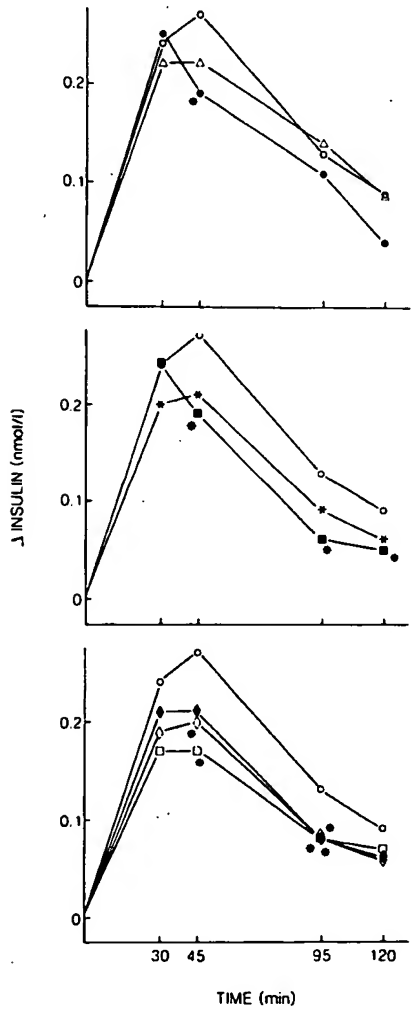

FIG 3. Insulin responses in healthy subjects to breakfast meals with white (top), coarse (middle), and high-soluble-fiber (bottom) breads. Each meal contained 50 g starch. WWB-mg is included as a rapid reference bread in all panels: O, WWB-mg; ●, WWB-tl; △, WWB-r; ■, CB-wwg; •, CB-sp; ◇, HSFB-ls; □, HSFB-ob; ◆, HSFB-mf. *Significantly different from WWB-mg by Wilcoxon test: $P < 0.05$ except for the comparisons with CB-wwg and HSFB-ob at 95 min ($P < 0.01$).

lipids, a fraction known to be less susceptible to α-amylase (35). However, lipids improve crumb texture by interacting with the gluten protein (41). The increased loaf volume and more porous and softer crumb of the WWB-mg product may have affected the glycemic and insulinemic responses. Although the WWB-mg appeared to disintegrate more completely in vitro when exposed to amylase and pepsin in the present study, there were no differences in the rate of appearance of dextrinized starch in the dialysate from monoglyceride addition. However, storage of bread, which is accompanied by an increased firmness of the crumb caused by retrogradation of amylopectine, has been shown to result in reduced blood glucose concentrations (42) despite the fact that not even considerable retrogradation of amylopectine, as occurs in stale bread, reduces the susceptibility of milled products to α-amylase (40). This may indicate effects on the rate of gastric emptying. Other reports indicate a relation between product porosity (degree of expansion) and the rate of α-amylolysis of cereal products (43). Further, according to Snow and O'Dea (3), a commercial white bread was much more rapidly digested by α-amylase in vitro than was a homemade bread. The difference might be related to texture because commercial bread making usually involves the addition of monoglycerides, stronger wheat varieties, and a more-intense mixing of the dough, all of which result in an increased bread volume and a softer crumb.

Actually, monoglyceride addition and/or textural differences may explain some of the discrepancies reported in in vitro digestion rates and/or glycemic responses between white wheat bread products (3, 11, 42). As pointed out by Bornet et al (11), GIs and insulinemic indexes range from 69% to 95% and 81% to 173%, respectively, when glucose is used as the carbohydrate reference. Interestingly, white breads with high GIs were of commercial origin whereas the low-GI breads were homemade. Bread has been preferred as a reference food (GI = 100%) (13, 44) because it is more palatable and avoids the possible problem of delayed gastric emptying of a glucose solution (44). However, as evident from the above discussion, GIs based on white wheat bread as a reference should not be compared unless the formula and baking process used are identical.

In a study in healthy subjects, bread rolls with a higher proportion of crust were reported to raise the capillary blood glucose more slowly than did a corresponding loaf (45). In the present study the glucose and insulin responses to bread rolls (WWB-r) were not significantly different from those to the white loaves (Figs 2 and 3, top) indicating that the crust-crumb relationship had no consequence on the rate of starch uptake.

The present study offers a comparison of meals with a whole-grain wheat bread (< 1 g soluble dietary fiber) and breads containing appreciable amounts of soluble dietary fiber incorporated at realistic amounts (3–3.9 g). The CB-wwg and the three HSFB products elicited similar glucose and insulin responses except for the unexpectedly high glucose response to the linseed bread (HSFB-ls) (Fig 2, bottom). Although in vitro data indicate a lowered rate of enzymic digestion for CB bread with intact kernels, a lowered rate of gastric emptying might be an additional mechanism (14). The low digestion rate is probably related to both a smaller product area as well as to the presence of cell walls encapsulating starch thereby restricting the availability to amylases. However, cell-wall components when present in a whole-meal wheat bread do not reduce the glycemic response (4). According to Jenkins et al (14) a clear distinction should be made between whole-meal and whole-grain breads with intact botanical structures. Inclosure of dense spaghetti cuts, as in the CB-sp product, also significantly reduced the GI and the inulinemic index. Actually, the observed GIs for CB-wwg (GI = 68) and CB-sp (GI = 79) were close to those expected in a composite product (GI = 71 and 77, respectively) when basing calculations on literature data (1) for whole-wheat grain (GI = 63), spaghetti (GI = 67), and white wheat bread (GI = 100), respectively.

Among the HSFBs, HSFB-ob tended to be most efficient in reducing the glucose and insulin responses. This might be partly related to the somewhat higher content of soluble fiber. Beta-glucans, a mixed 1, 3 and 1, 4 polysaccharide, constitutes the major soluble-fiber component in oats (29) and gives a highly viscous solution when dissolved in water. Isolated preparations of oat gum (80% of β-glucan) reduce the glucose and insulin responses to glucose drinks by the same magnitude as guar gum (46). Obviously, the positive effect of oat β-glucan remains also after proofing and baking. Oat-based meals (scones and porridge) also evoked smaller glucose and insulin responses than did wheat-based meals (5). The reduced insulin response to the HSFB-ls indicated that the viscous fiber polysaccharides of linseed also affected the metabolic response to starch. The reduction in insulin was important and of the same magnitude as that obtained with HSFB-ob. However, the lack of effect on glucose response by HSFB-ls complicates interpretation. Addition of linseed increased bread volume, porosity, and crumb softness substantially. Obviously, the fiber polysaccharides and/or the lipids in linseed functioned as bread improvers, resulting in textural characteristics that may have increased the digestion rate and thus counteracted the absorption-delaying effect of the viscous-fiber components. The mechanisms for the inconsistent data with oat bran and linseeds are interesting and deserve further attention. It is possible that linseed reduces insulin secretion by a mechanism other than by lowering the rate of starch uptake, ie, by reducing gastric inhibitory polypeptide (GIP) responses or periferial insulin resistance. Hence, in addition to a lowered rate of glucose uptake, a lowered GIP response was suggested to explain the prominent reduction in insulin response to intact vs milled rice (47).

In most studies reporting glycemic responses to starchy foods, comparisons are made with equal amounts of digestible carbohydrate. This offers a possibility to rank various foods by rates of starch digestion and absorption. However, ranking of foods based on equal amounts of digestible starch may not always be practical because the content of digestible carbohydrate may differ greatly. Actually, the beneficial effects of high-fiber meals may be due partly to the reduced content of digestible carbohydrates (48, 49). According to Heinonen et al (48), a high-fiber bread with a starch content of 80% of that in white bread elicited an area under the glucose curve that was only 67% of that after the white bread in diabetic subjects. When the areas were calculated per gram of absorbable carbohydrate, all statistical differences disappeared. The starch in the present high-fiber breads was replaced by dietary fiber and, especially in CB-wwg and HSFB-ob, with substantial amounts of water. Consequently, the content of digestible carbohydrate, calculated on a wet weight basis, in HSFB and CB-wwg amounted to only 64–75% of that in the white wheat loaves. In addition, all HSFBs and CB-wwg showed higher satiety scores compared with the WWBs immediately after the meal was finished, which indicates that incorporation of dietary fiber may restrict the voluntary food intake. Consequently, in practice the slower rates of digestion and absorption of the starch, the lower starch content, as well as a higher satiety-promoting capacity should all contribute to a re-

duced glycemic response to whole-grain bread or to bread rich in soluble dietary fiber components. It is concluded that inclosure of intact wheat grains or oat bran show the most promising potential for developing lente bread. The whole-grain wheat bread also contained the highest level of RS.

We thank Anders Drews for his invaluable technical assistance. Information concerning raw materials and the baking process was provided by Gunnar Hall and Christer Johnsson, Nordmills AB, Malmö, Sweden. The bread products were skillfully baked by Arne Bjager, Nordmills AB, Malmö, Sweden.

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APPENDIX E

Glycemic index of processed wheat products[1,2]

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ABSTRACT Our aim was to determine the in vivo glycemic and insulin responses and in vitro starch digestibility of seven processed wheat products (shortbread biscuits, custard, quick-cooking wheat, wholemeal bread, water biscuits, puffed wheat, and puffed crispbread). The degree of starch gelatinization in the foods was measured. Fifty-gram carbohydrate portions of the foods were fed to eight volunteers after an overnight fast. The calculated glycemic indices (GI) (mean ± SEM) ranged from 43 ± 10 for custard to 81 ± 9 for puffed crispbread. Insulin responses paralleled the glycemic responses. The GI correlated positively with the percentage of starch digested in vitro ($p < 0.05$). The degree of starch gelatinization ranged from 0.4 to 60% and correlated positively with the percentage starch digested in vitro ($p < 0.05$). Differences in the glycemic and insulin responses to wheat products may be explained in part by the extent of processing and the degree of gelatinization achieved. *Am J Clin Nutr* 1987;46:631–5.

KEY WORDS Glycemic index, food processing, starch gelatinization, starch digestibility

Introduction

Over the past few years it has become increasingly clear that different starchy foods are digested and absorbed at different rates (1). Cooking and gelatinization of starch (ie, swelling of the granules in the presence of heat and water) increase its susceptibility to enzymic degradation in vitro (2) and its availability for digestion and absorption in the small intestine (3, 4). We showed previously that modern methods of food processing such as thermal extrusion render starch in foods based on corn, rice, and potato more digestible than home-cooking methods such as boiling or baking (5). The aim of this study was to examine the effects of processing on digestibility of wheat products. In vitro starch digestibility and in vivo glycemic and insulin responses were measured in seven processed wheat products. The extent to which the results were related to the degree of starch gelatinization also was examined. Our hypothesis was that new methods of food processing such as thermal extrusion and puffing would render starch more gelatinized and therefore more digestible.

Materials and methods

The wheat products studied were chosen to represent a range of commercial processing techniques. They were wholemeal bread, quick-cooking wheat, puffed wheat, shortbread biscuits, puffed crispbread, water biscuits, and custard prepared from wheat starch. A description of the products and the processing methods involved is shown in **Table 1**. The nutrient composition of the foods was obtained from Paul and Southgate (9) and the manufacturers (**Table 2**). Available carbohydrate (sugar and starch) was measured directly by digesting the foods with amyloglucosidase and measuring the sugars by high-pressure liquid chromatography (HPLC) (10, 11).

In vitro study

All the foods other than quick-cooking wheat, bread, and custard were prepared by light grinding using a food mill (2 mm screen). Quick-cooking wheat was cooked and then lightly ground with a mortar and pestle to simulate mastication. The wholemeal bread was finely crumbed. Custard was prepared from a recipe (Table 1) and required no further treatment. The method used to measure in vitro starch digestibility was a modification of the method of Jenkins et al (1). One-gram carbohydrate portions were mixed with 3 mL porcine pancreatin (1% wt/vol, Sigma, grade VI, St Louis, MO), 2 mL human saliva (freshly pooled and then centrifuged, 1300 × *g* for 10 min at 10 °C) and 10 mL phosphate buffer (pH 6.9). After 4-h incubation, the food and enzyme mixture was heated (80 °C, 5 min) to inactivate amylase and then was centrifuged (10 000 × *g*, 5 min). The supernatant was filtered (microporous filters, 25 mm, 0.45 μm, Amicon, Lexington, MA) and analyzed for sugars by HPLC (Varian, Walnut Creek, CA). The percentage of starch digested was calculated after taking into account the free sugars in the food and the potential trapping of sugars by the food (5). The

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TABLE 1
Description of the wheat products studied

Food	Manufacturer	Processing
Custard (based on wheat starch)	Fielders (Sydney, Australia)	Prepared from commercial wheat starch (20 g), egg (60 g), milk (360 mL), and sugar (30 g).
Quick-cooking wheat	White Wings (Sydney, Australia)	Whole-wheat grains are physically treated to induce numerous small fissures, which allow rapid hydration and short cooking times (6).
Shortbread biscuits	Arnotts (Sydney, Australia)	Commercial baking of high-fat, high-sugar, low-moisture dough (7).
Wholemeal bread	Tip Top (Sydney, Australia)	Commercial baking of a high-moisture dough
Water biscuits	Arnotts (Sydney, Australia)	Commercial baking of low-fat, low-sugar, high-moisture dough (7).
Puffed wheat (ready-to-eat breakfast cereal)	Sanitarium (Sydney, Australia)	Whole-wheat grains are heated at high pressure with steam, followed by rapid release of pressure. Sudden expansion and release of water vapor blows up the grains to several times their original size (puffed).
Puffed crispbread	Westons (Sydney, Australia)	Produced by thermal extrusion (8). A high-moisture dough is cooked under pressure, extruded through a die and puffed with the sudden release of pressure (*see* puffed wheat above).

experiments were repeated three times for each food (variation was < 10%). One-way analysis of variance (ANOVA) and Fisher's test (12) were used to determine the significance of differences between the foods.

Measurement of the degree of starch gelatinization

Foods were prepared as for the in vitro starch digestion experiments. The method used to measure the degree of gelatinization of the foods was based on starch-iodine complexing reactions (13). Average values for three experiments were calculated (variation was < 10%) and the results were expressed as a percentage. This method is unsuitable for bread that is not oven fresh because retrogradation of the starch (aligning of the amylose molecules) occurs soon after removal from the oven. Hence the degree of gelatinization of bread was taken from published reports, which used the same method on oven-fresh samples (13).

In vivo study

Eight healthy volunteers took part in the study (four men and four women aged 21–32 y with body mass index between 18 and 26 kg/m^2). They had fasting capillary plasma glucose levels within the normal range (4.2–5.6 mmol/L) and gave normal 2-h responses to the glucose tolerance test (< 8.9 mmol/L). The subjects took 50 g available carbohydrate portions of each food (Table 1) or glucose in random order on separate mornings after a 10-h overnight fast. For individual subjects, the tests were ~1 wk apart. Foods were consumed over 10 min with 450 mL tea (400 mL black tea and 50 mL milk) giving an average meal volume of 560 mL (range 485–680 mL). Finger-prick capillary blood samples were taken at 0 (fasting), 15, 30, 60, 90, 120, 150, and 180 min after the meal was commenced. Hands were placed in a 45 °C water bath for at least 2 min before puncturing with an Autolet device (Owen Mumford Ltd, Woodstock, UK) using Autoclix lancets (Boehringer Mannheim, Mannheim, FRG). Blood (500 μL) was collected into 1 mL tubes with 0.4 mg EDTA (Ajax Chemicals, Sydney, Australia) and plasma was removed after centrifugation (10 000 × *g*, 1 min) and stored at −80°C before analysis. Glucose was assayed by the glucose hexokinase method using the Glucose Rapid Centrifichem System (Roche Diagnostica, Basle, Switzerland) and insulin by double antibody radioimmunoassay (Bio-RIA, Montreal, Canada). The glycemic

TABLE 2
Weight of meal and nutrient content of 50 g available carbohydrate portions of the seven wheat products studied*

Food	Weight of meal (g)	Protein (g)	Water (g)	Fat (g)	Sugars (g)	Dietary Fiber (g)	Energy (kJ)	Source
Custard	303	13.4	258.0	14.2	34.0	0	1551	(9)
Quick cooking wheat (after preparation)	161	4.9	†	3.2	†	17.6	1002	Manufacturer
Shortbread biscuits	85	4.9	4.3	19.0	10.9	1.8	1688	Manufacturer
Bread	156	15.2	62.4	4.2	2.3	10.1	1326	Manufacturer
Water biscuits	71	6.8	3.2	5.7	1.7	2.3	1335	Manufacturer
Puffed wheat	76	10.8	1.9	1.0	1.7	11.7	1055	(9)
Puffed crispbread	71	7.9	3.2	2.5	0.5	2.9	1157	Manufacturer

* Available carbohydrate (starch plus sugars) was measured directly.
† No figure available.

TABLE 3
Glycemic index, insulin index, starch digestibility in vitro and degree of gelatinization in the seven wheat products studied

Food	Glycemic index Mean (SEM)	Insulin index Mean (SEM)	Starch digested in vitro	Degree of gelatinization
			%	%
Custard	43 (10)	89 (19)	78	42
Quick-cooking wheat	54 (11)	45 (8)	53	11
Shortbread biscuits	64 (8)	80 (9)	58	0.4
Wholemeal bread	77 (9)	102 (23)	76	60
Water biscuits	78 (11)	105 (22)	74	1.5
Puffed wheat	80 (11)	79 (13)	105	54
Puffed crispbread	81 (9)	99 (16)	97	50

index (GI) and insulin index (II) of each food were calculated for each subject with the following equations:

$$GI = \frac{\text{incremental area under 2-h plasma glucose curve for food}}{\text{incremental area under 2-h plasma glucose curve for 50 g glucose}} \quad (1)$$

$$II = \frac{\text{incremental area under 2-h plasma insulin curve for the food}}{\text{incremental area under 2-h plasma insulin curve for 50 g glucose}} \quad (2)$$

Results were expressed as mean ± SEM. Two-way analysis of variance (ANOVA) was used to determine the significant heterogeneity and the Newman-Keuls' test (12) to compare differences between the foods. Linear regression was used to measure correlation between variables.

The study was approved by the Medical Ethical Review Committee of the University of Sydney.

Results

In vitro study

The percentage starch digested in vitro ranged from 53% in quick-cooking wheat to 105% in puffed wheat (**Table 3**) with significant differences among the foods ($p < 0.01$, ANOVA). The percentage of starch digested in puffed wheat and puffed crispbread was significantly higher than in the other foods ($p < 0.001$, Fisher's test) while the digestibility of shortbread biscuits and quick-cooking wheat was significantly lower than the other foods ($p < 0.001$, Fisher's test).

In vivo study

The mean plasma glucose and insulin response curves to the foods are shown in **Figures 1 and 2.** The GIs ranged from 43 ± 10 for custard to 81 ± 9 for puffed crispbread (Table 3) with significant differences among the foods ($p < 0.005$, ANOVA). The GI of custard was significantly lower than that of puffed crispbread, puffed wheat, water biscuits, and bread ($p < 0.05$, Newman-Keul's test).

The IIs ranged from 45 ± 8 for quick-cooking wheat to 105 ± 22 for water biscuits (Table 3) with significant differences among the foods ($p < 0.025$, ANOVA). The II of quick-cooking wheat was significantly lower than that of water biscuits, bread, puffed crispbread, and custard ($p < 0.05$, Newman-Keul's test).

There was a significant positive correlation between the GI and the II of the foods ($r = 0.61$, $p < 0.001$, $n = 64$). Apart from the sugar content of the foods, which correlated negatively with the GI ($r = 0.81$, $p < 0.05$), the nutrient composition (protein, fat, fiber, and energy content) of the foods did not appear to be related to either the GI or the II. Excluding custard, the GI correlated with the percentage starch digested in vitro ($r = 0.86$, $p < 0.05$). The glycemic response to custard was much lower than its in vitro starch digestibility.

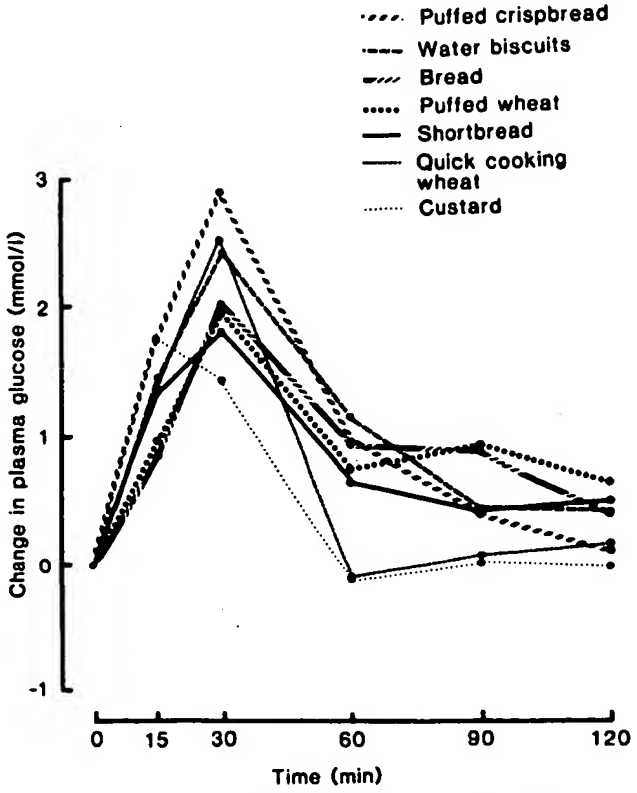

FIG 1. The mean glycemic response to each of the foods.

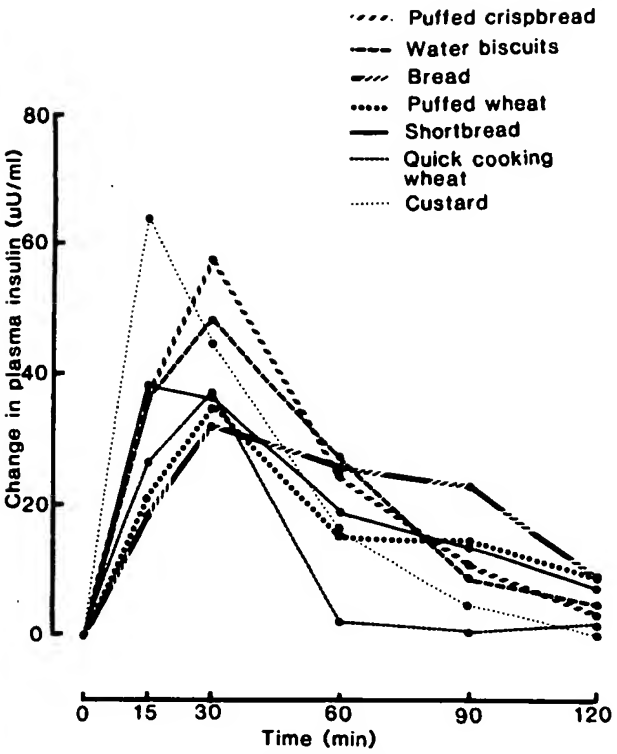

FIG 2. The mean plasma insulin response to each of the foods (1 μU/mL = 7.1 pmol/L).

Degree of gelatinization of starch

The degree of gelatinization ranged from 0.4% for shortbread biscuits to 60% for bread (Table 3). A significant positive correlation was found between the degree of starch gelatinization of the wheat products and the percentage starch digested in vitro ($r = 0.73$, $p < 0.05$). The correlation between starch gelatinization and GI did not reach statistical significance ($r = 0.61$, $0.05 < p < 0.1$).

Discussion

This study has shown that modern methods of processing affect the digestibility of wheat starch both in vitro and in vivo. The results also suggest that gelatinization may be one of the factors that determines the rate of starch digestion and hence the subsequent glycemic response.

Starch digestibility is increased when foods are subjected to conditions that increase the accessibility of starch to amylase. The granular structure of starch may be destroyed mechanically (eg, by grinding) and by heat and water (gelatinization). During gelatinization, the starch granules absorb water and swell, eventually rupturing to expose the individual starch molecules (14). The extent or degree of starch gelatinization is dependent on moisture availability, time, temperature, and pressure (15) and generally can be explained by the method of processing to which the foods have been subjected.

Among the foods examined in this study, puffed wheat and puffed crispbread are produced by methods that require a high prebaking water:flour ratio (Table 1) (16). This allows starch granules to completely gelatinize under the high temperatures (100–250 °C) and high pressures (2–20 MPa) but short cooking times (5–120 s) involved (14). The intense mechanical treatment caused by the rotating screws that mix and transport the ingredients before extrusion partly destroys the structure of the starch granules, further increasing starch digestibility. The high GI and in vitro starch digestibility of puffed wheat and puffed crispbread found in this study are therefore likely to be related to the type of processing and the high degree of gelatinization achieved. Other studies also have reported that extruded products have greater digestibility in vitro and in vivo than boiled or baked products (5, 17, 18).

At the other extreme are sweet biscuits such as shortbread, which have a low prebaking water:flour ratio and moderate temperatures during baking (13). This results in low levels of starch gelatinization, a consequence of which appears to be significantly lower starch digestibility in vitro and in vivo. The presence of sucrose (19) and fat (20) also is known to decrease starch gelatinization and both are high in shortbread biscuits.

The positive correlation between in vitro starch digestibility and degree of gelatinization is not altogether surprising. There are several methods of measuring degree of gelatinization, one of which utilizes an in vitro amylase digestion system (21). Hence the present methods of measuring in vitro rate of digestion of food are to a large extent also measuring the degree of gelatinization. But factors other than gelatinization also contribute to the overall digestibility of starch. The whole-grain physical structure of quick-cooking wheat would tend to decrease the accessibility of amylase to its starch (22) and explain the relatively low digestibility of this product. The amylase inhibitor activity in the germ fraction of wheat (2) also may be higher in quick-cooking wheat. The digestibility of starch may be decreased by nonenzymic browning (Maillard) reactions (23). The degree of browning depends on process times and temperatures, the presence of moisture, and, in particular, reducing substances such as sugars (24, 25). These factors may partly explain different digestibilities in vivo and in vitro between shortbread biscuits and water biscuits. Shortbread biscuits have higher levels of nonenzymic browning because of their high sugar and low prebaking moisture content.

The higher digestibility of custard in vitro compared with its low glycemic response may be explained by its high fat content, which decreases the rate of gastric emptying and its high fructose content (from hydrolysis of sucrose), which produces a lower glycemic response than the equivalent amount of glucose.

Comparison of our GI results for bread with other studies indicates good reproducibility among different research groups. Wholemeal bread was found to have a GI of 72 in one study (26) and 75 in another (27) compared with 77 in this study. Unlike Collier et al (28), we found that insulin responses do run parallel to the glycemic responses.

It is possible that results of Collier et al are related to the large amount of fat given in their study compared with the moderate amounts in this study.

Although there were no home-cooked foods in this study, the products with the least degree of processing (custard, quick-cooking wheat, and shortbread biscuits) have lower GIs and/or in vitro digestibility than those produced by more modern methods such as puffing and thermal extrusion (puffed wheat and puffed crispbread). The findings therefore add more support to the conclusion of our previous study of rice, corn, and potato products (5), viz, the more processed a food is, the higher the glycemic response it will produce. Although pasta may appear at first to be an exception because its GI has been found to be low (26), its manufacture is fairly simple involving relatively little mechanical disruption of the starch granule. The starting product is semolina, which is milled from very hard wheat such as durum (7). The stiff dough made by mixing semolina with water is pushed under pressure through a die and then dried.

In summary, this study indicates that there are twofold differences in the glycemic and insulin responses to wheat products and that such differences may be explained in part by the severity of processing and the degree of gelatinization achieved. The findings may provide useful information not only for diabetic diets but also for applications that include exercise performance, weight reduction, and dental caries prophylaxis.

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APPENDIX F

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Consumption of the slow-digesting waxy maize starch leads to blunted plasma glucose and insulin response but does not influence energy expenditure or appetite in humans

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Abstract

Limited research in humans suggests that slowly digestible starch may blunt the postprandial increase and subsequent decline of plasma glucose and insulin concentrations, leading to prolonged energy availability and satiety, compared to more rapidly digestible starch. This study examined the postprandial metabolic and appetitive responses of waxy maize starch (WM), a slow-digestible starch. It was hypothesized that the waxy maize treatment would result in a blunted and more sustained glucose and insulin response, as well as energy expenditure and appetitive responses. Twelve subjects (6 men and 6 women) (age, 23 ± 1 years; body mass index, 22.2 ± 0.7 kg/m^2; insulin sensitivity [homeostatic model assessment], 16% ± 2%; physical activity, 556 ± 120 min/wk) consumed, on separate days, 50 g of available carbohydrate as WM, a maltodextrin-sucrose mixture (MS), or white bread (control). Postprandial plasma glucose and insulin, energy expenditure, and appetite (hunger, fullness, desire to eat) were measured over 4 hours. Compared to control, the 4-hour glucose response was not different for MS and WM, and the 4-hour insulin response was higher for MS ($P < .005$) and lower for WM ($P < .05$). Compared to MS, WM led to lower 4-hour glucose and insulin responses ($P < .001$). These differences were driven by blunted glucose and insulin responses during the first hour for WM. Postprandial energy expenditure and appetite were not different among treatments. These results support that WM provides sustained glucose availability in young, insulin-sensitive adults.

Keywords: Starch; Glycemic index; Glucose; Insulin; Appetite; Polysaccharide; Humans
Abbreviations: Au, arbitrary units; ANOVA, analysis of variance; AUC, area under the curve; CHO, carbohydrate; HOMA, homeostatic model assessment; MS, maltodextrin-sucrose mixture; WM, waxy maize starch.

1. Introduction

Researchers have begun to examine the utility of using slowly digested starch to influence the postprandial blood glucose and insulin concentrations leading to prolonged energy availability [1]. One application of this might be to improve exercise performance, delay fatigue, and increase

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physical endurance through extended glucose release [2]. Other applications could be to prolong satiety and improve diabetes management [3].

Starch can be separated into 3 categories based on its digestibility, which is determined by the rate that glucose is released from the starch and then absorbed [4]. Rapidly digestible starch, such as cooked/pregelatinized starch, is enzymatically digested in vitro within 20 minutes. Resistant starch is the residual starch and degradation by-products not digested or absorbed in the small intestine. Resistant starch is fermented by bacteria when it reaches the large intestine and is not a direct source of energy to the body but does contribute energetically through production and absorption of short chain fatty acids [5]. Slowly digested starch (which includes the uncooked cereal starches maize, waxy maize, barley, wheat, and rice) is digested enzymatically in vitro between 20 and 120 minutes. In the case of maize starches, the progress of digestion is from the inside of the starch structure to the outside (inside-out starch digestion) because of the presence of surface pores and channels within the granule [5,6].

There are few reports of data regarding the effects of slowly digestible starch on glucose tolerance, energy expenditure, and appetite. The ingestion of 35 g of available carbohydrate as maize starch or waxy maize starch (WM) (slowly digestible starches) resulted in a smaller increase and longer sustained rise in plasma glucose compared to maltodextrin (a rapidly digestible starch) [2].. The ingestion of a mixture of tapioca and maize starch (slowly digestible starch) lowered the incremental area under the curve for insulin and tended to lower the glucose profile compared to the ingestion of a rapidly digesting starch (a waxy maize-derived starch) [1]. In young healthy women, a meal containing a slowly digestible WM resulted in lower peak concentrations of plasma glucose and insulin compared with a meal containing a rapidly digestible maize starch [5]. In young men, the consumption of uncooked cornstarch (a slowly digested starch) led to blunted plasma glucose and insulin responses. The area under the curve for the slowly digesting starch was lower during the first 120 minutes, but there were no difference after 120 minutes compared to consuming glucose [8]. Similar results were found in a study comparing slowly digesting barley kernels with a white bread control [9]. Concerning appetite, the ingestion of slowly digested barley kernels is reported to cause greater satiety over a 3-hour period compared to white bread. In vitro research documents that waxy maize is a slowly digested starch [6]. We are not aware of any published research examining the metabolic, energy expenditure, and appetitive responses of WM in humans. Thus, the primary purpose of this study is to examine the effects of waxy maize on postprandial plasma insulin and glucose, and secondarily, whole-body energy expenditure and appetite in men and women. We hypothesized that the waxy maize treatment would result in a blunted, more prolonged postprandial glucose and insulin response compared to a maltodextrin-sucrose mixture (MS) (rapidly digestible carbohydrate) or white bread (glycemic control). We also hypothesized that the WM would result in a more prolonged appetitive response compared to the other treatments.

2. Methods and materials

2.1. Subjects

Potential participants were recruited from public advertisements placed in the local newspaper, in local businesses, and in buildings on the Purdue University campus. Study inclusion was based on the following criteria: (1) men and women aged 18 to 29 years; (2) body mass index between 18.5 and 24.9 kg/m^2; (3) body fat of less than 27% for women and less than 20% for men; (4) not dieting; (5) weight stability ± 2 kg within the last 3 months; (6) nonsmoker; (7) clinical normalcy for indices of liver and kidney functions and complete blood count (non-anemic); (8) fasting plasma glucose of less than 6.1 mmol/L; and (9) perform at least 1 h/d of aerobic activity on at least 4 d/wk (240 min/wk) for the past 3 months. All women recruited into the study were taking oral or hormonal contraceptives for the past 6 months. Inclusion criteria were chosen based on army criteria for a physically fit individual.

Subject characteristics are shown in Table 1. There were 187 total contacts, of which 45 met the initial telephone screening criteria. Fifteen individuals (7 men, 8 women) met all study criteria and began the study. Twelve subjects (6 men, 6 women) completed all testing days.

All study procedures were approved by the Purdue University Biomedical Institutional Review Board, and all subjects were informed of the purpose, procedures, and potential risks of the study before signing the informed consent document. Each subject received monetary compensation for participation. A clinical trials registration number is not provided because the study was conducted before the requirement for registration was established.

Table 1
Subject characteristics

	Subjects (n = 12)	Men (n = 6)	Women (n = 6)
Age (y)	23 ± 1	21 ± 1	25 ± 1
Height (cm)	172 ± 2	176 ± 2	168 ± 2
Weight (kg)	65.0 ± 2.1	67.0 ± 2.5	62.5 ± 3.4
BMI (kg/m^2)	22.2 ± 0.7	21.6 ± 1.0	22.2 ± 0.9
Body fat (%)	17.0 ± 2.2	10.8 ± 1.3	23.2 ± 1.8
Fat mass (kg)	11.0 ± 1.5	7.3 ± 1.1	14.8 ± 1.7
Fat-free mass (kg)	53.7 ± 2.5	59.9 ± 2.0	47.9 ± 2.0
Fasting glucose (mmol/L)	4.8 ± 0.1	5.1 ± 0.1	4.6 ± 0.2
Aerobic activity (min/wk)	556 ± 120	775 ± 207	337 ± 44
HOMA (%)	16 ± 2	13 ± 2	19 ± 2

Data are expressed as means ± SEM. BMI indicates body mass index.

2.2. Experimental design

This study was a randomized, crossover design with 3 treatments. Each subject was tested on 3 separate days over a period of 5 weeks with one or more days between testing days. The clinical phase of this study was accomplished between July 2006 and December 2006.

Upon arrival at the laboratory after a 10-hour period of fasting, the subject was asked to void and then rest on a bed in a supine position. A catheter was placed in an antecubetal vein of the nondominant arm and kept patent for the next 5 hours using a saline drip. Twenty and 30 minutes after the catheter was inserted (denoted as minutes −40 and −30 on the timeline in Fig. 1), baseline blood samples were taken and appetite and mood state questionnaires were completed. Resting energy expenditure (fasting state) was then measured using an indirect calorimeter for the next 30 minutes. The subject was then randomly given 1 of 3 treatments (Table 2). Blood sampling and appetite and mood state questionnaires were repeated and resting energy expenditure (postprandial state) was measured over the next 4 hours.

Table 2
Experimental treatments

	White bread control	Maltodextrin	Waxy maize
General characteristics:			
Energy (kJ/treatment)	1151	875	921
Protein (g/treatment)	7.5	0	0
Available CHO (g/treatment)	50	50	50
Fat (g/treatment)	3.8	0	0
Weight (g/treatment)	103.7	110	110
Viscosity at 60 rpm (centipoise)	N/A	215	224
Formulation	N/A	Maltodextrin = 38.2g Sucrose = 11 g Mira Gel® 463 Tate and Lyle = 4.8g Water = 55.6 g	Mira Gel® 463 Tate and Lyle = 2.2g Waxy maize = 51.7 g Water = 55.1 g Sucralose = 0.3 g

CHO indicates cholesterol; N/A, not applicable.

2.3. Treatments

The subjects consumed 50 g of available carbohydrates as faster-digesting, 78%:22% MS or slower-digesting, uncooked WM (Table 2). Both products were incorporated into a gel matrix composed of nonstarch hydrocolloids. Waxy maize starch was provided by Tate & Lyle (Decatur, Ill). Water was added to the gel mixtures so that the mixtures were in a drinkable form. These mixtures were compared to a white bread control (Wonder-Classic brand white bread, with the crust removed [Interstate Bakeries Corporation; Kansas City, Mo]), which was frozen and then defrosted before each test day. The weight of each treatment to be consumed was calculated based on the amount of

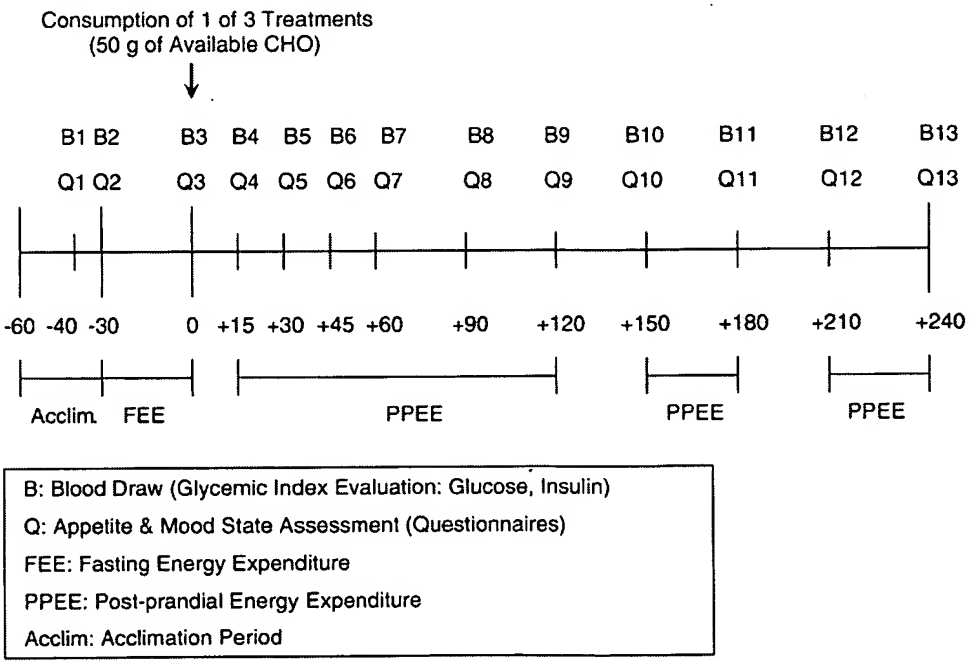

Fig. 1. Testing day protocol. Values from −60 to +240 represent the time in minutes relative to when the subject started to consume one of the test foods.

available carbohydrate. In the case of the white bread control, available carbohydrate was the "carbohydrate, by difference" value from the USDA National Nutrient Database for Standard Reference [10]. Viscosities of the test meals were equalized, using a Brookfield Viscometer (Brookfield Engineering Laboratories; Middleboro, Mass) to ensure that viscosity did not affect the differences in glycemic response between the products. Water was provided to the participants for each treatment so that the amount of water consumed was equal across treatments, including the water added to treatments to decrease viscosity. This totaled 240 mL of water with each treatment.

2.4. Body composition

During screening, each subject's body volume and mass were measured once using a plethysmography system (BOD-POD, Life Measurement Instruments, Inc, Concord, Calif); whole-body density (mass/volume) was determined, from which estimates of fat mass and fat-free mass were made. Standing height (with the subjects wearing socks) was measured using a stadiometer. Body mass index was calculated from body mass and height (kilogram per square meter).

2.5. Physical activity

During screening, each subject completed a validated physical activity recall questionnaire addressing all moderate to vigorous aerobic activity performed over the previous 3-month period [11].

2.6. Resting energy expenditure

Fasting- and postprandial-state whole-body oxygen uptake and carbon dioxide production were measured using an indirect calorimeter (MedGraphics Cardiopulmonary Diagnostics Systems; MedGraphics Corporation, St Paul, Minn) during the times indicated in Fig. 1. Energy expenditures at these times were estimated using the Weir Equation [12].

2.7. Appetite

A visual analog scale questionnaire [13] was used to assess the appetitive perceptions of hunger, fullness, and desire to eat at the times denoted in Fig. 1. A 13-point linear scale with end anchors of "not at all" and "extremely" was used to assess each perception. The participants circled the vertical dash along the horizontal line corresponding to their feelings at that moment. The results are reported using arbitrary units.

2.8. Blood sampling, and glucose and insulin analyses

Thirteen blood samples were taken during each testing period (Fig. 1). The samples were collected in test tubes containing EDTA and centrifuged at 3000 rpm and −4°C for 15 minutes. The plasma was then extracted from the tube and aliquots stored in microcentrifuge tubes at −80°C for future analyses. Plasma glucose concentration was measured by enzymatic colorimetry, using an oxidase method on a COBAS Integra 400 analyzer (Roche Diagnostic Systems, Indianapolis, Ind). Plasma insulin concentration was measured by an electrochemiluminescence immunoassay method on the Elecsys 2010 analyzer (Roche Diagnostic Systems).

2.9. Calculations and statistical analyses

Areas under the curve (AUC) for postprandial appetite, energy expenditure, plasma glucose, and plasma insulin were calculated using the trapezoidal rule [13]. Glycemic index was calculated over a 2-hour period (traditional calculation) and a 4-hour period (total time of testing) using the following equation [15]:

$$GI = [(\text{postprandial incremental AUC of the test treatment} / \text{postprandial incremental AUC of the white bread control}) \times 100] / 1.4^{\dagger} \quad (1)$$

The homeostatic model assessment (HOMA), a measure of insulin sensitivity, was calculated using the following equation and conversion factors [16]:

$$\text{HOMA (\%)} = \text{fasting insulin (pmol/L)} \times \text{fasting glucose (mmol/L)} / 57.02 \quad (2)$$

Insulin: 1 μU/mL = 7.175 pmol/L
Glucose: 1 mg/dL = 0.0055 mmol/L

All values are expressed as means ± SEM. Glucose data from one subject were excluded because their baseline concentration was inexplicably high for one of the trials. Repeated-measures analysis of variance (ANOVA) with least significant difference pairwise comparisons were performed on all of the parameters and $P < .05$ considered statistically significant. A power calculation was performed using a repeated-measures ANOVA that showed greater than 95% power to detect differences in 4-hour plasma glucose and insulin concentrations between treatments. Statistical analyses were performed using SPSS (Version 12.0; SPSS, Chicago, Ill).

3. Results

3.1. Glycemic index

The glycemic index of each treatment is shown in Table 3. At 2 and 4 hours, MS was not different and WM was lower ($P < .05$) compared to the white bread control. WM was lower than MS at 2 and 4 hours ($P < .005$).

† 1.4 is the correction factor when using white bread as the control instead of glucose [14].

Table 3
Glycemic index after the consumption of the study treatments in 12 subjects

	Calculated glucose control	White bread control	Maltodextrin	Waxy maize
Glycemic index (2-h)	100 ± 0[a] (100)	71 ± 0[a] (71)	163 ± 37[a] (106)	63 ± 11[b] (58)
Glycemic index (4-h)	100 ± 0[a] (100)	71 ± 0[a] (71)	127 ± 27[a] (89)	60 ± 11[b] (64)

Values are means ± SEM (median). Values within a row with a different superscript letter differ ($P < .05$). Glycemic index was calculated over both a 2- and 4-hour period, using the equation as follows: GI = [(postprandial incremental AUC of the test treatment/postprandial incremental AUC of the white bread control) × 100]/1.4. The correction factor 1.4 was used because of the use of white bread as a control rather than glucose [14].

3.2. Glucose

Consumption of the white bread control led to a gradual rise in plasma glucose, reaching a peak concentration of 5.91 ± 0.16 mmol/L at 60 minutes, followed by a gradual lowering toward baseline over the 4-hour period (Fig. 2). The postprandial rise in plasma glucose resulting from MS was higher and faster, with a peak concentration of 6.80 ± 0.28 mmol/L at minute 45. The glucose response of WM was comparable to the white bread control. The peak glucose concentration of WM was 5.83 ± 0.39 mmol/L at minute 60. The composite glucose AUC response over the 4-hour period was not different between the white bread control (84 ± 12 mmol/L per 240 minutes) and the other treatments (Fig. 2). However, the WM led to lower glucose AUC (62 ± 9 mmol/L per 240 minutes) vs MS (121 ± 16 mmol/L per 240 minutes; $P < .001$). Comparable results were shown for the glucose AUC response during the first 60 minutes: control, MS, and WM, 36 ± 6[ab], 78 ± 11[a], and 25 ± 4[b] mmol/L per 60 minutes, respectively (different superscript letters differ, $P < .05$). There were no differences in hourly glucose AUC responses among the treatments during hours 2, 3, and 4.

3.3. Insulin

Ingestion of the white bread control led to a gradual rise in plasma insulin, reaching a peak concentration of 121 ± 25 pmol/L at 45 minutes (Fig. 3). Compared to the white bread control, MS led to a greater and faster increase in plasma insulin, reaching a peak concentration of 200 ± 58 pmol/L at 30 minutes, and WM led to lower peak (74 ± 18 pmol/L) that occurred at 45 minutes. The insulin AUC responses over 4 hours were different among the 3 treatments. Compared to control (9.13 ± 1.25 nmol/L per 240 minutes), the MS response was higher (13.43 ± 2.09 nmol/L per 240 minutes; $P < .005$) and the WM response lower (5.98 ± 1.17 nmol/L per 240 minutes; $P < .05$). The largest difference among treatments occurred during the first 60 minutes. Specifically, the insulin AUC response of the first 60 minutes was lower after the WM treatment (2.44 ± 0.56 nmol/L per 60 minutes) compared to MS (8.61 ± 1.68 nmol/L per 60 minutes; $P < .001$) and tended to be lower than the control (3.96 ± 0.66 nmol/L per 60 minutes; $P = .100$), although glucose response profiles were similar. There were no differences in hourly insulin AUC responses among the treatments during hours 2

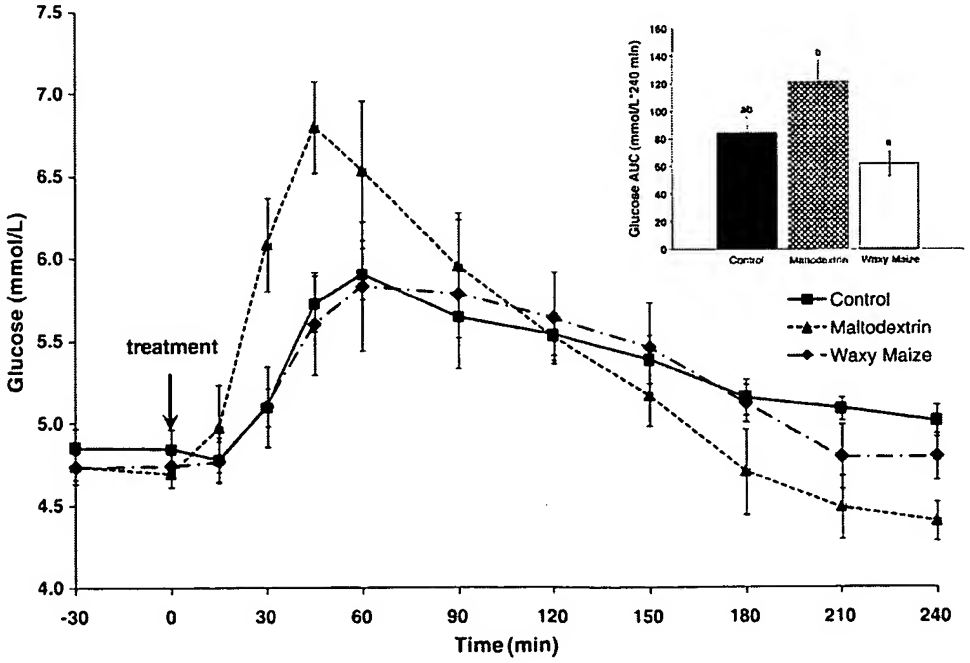

Fig. 2. Glucose response over 4 hours after the consumption of the study treatments in 11 subjects. Area under the curve graph for the glucose response where different letters represent statistically significant differences ($P < .05$).

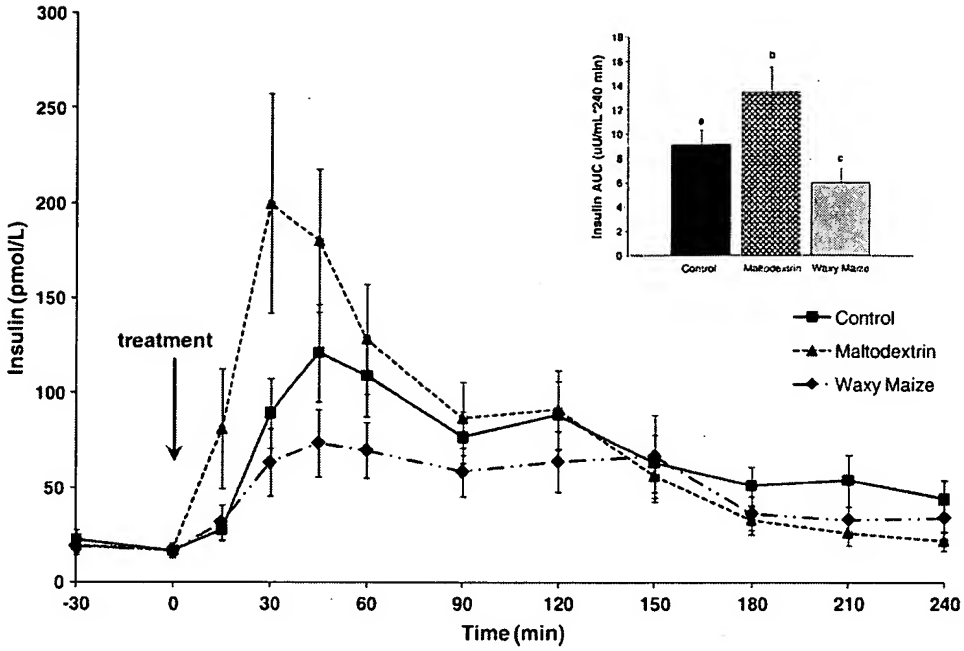

Fig. 3. Insulin response over 4 hours after the consumption of the study treatments in 12 subjects. Area under the curve graph for the glucose response where different letters represent statistically significant differences ($P < .05$).

and 3; however, in hour 4, compared to the control (1.06 ± 0.23 nmol/L per 240 minutes), both MS (0.31 ± 0.14 nmol/L per 240 minutes) and WM (0.52 ± 0.14 μU /mL per 60 minutes) were lower, but MS was not different from WM.

3.4. Postprandial energy expenditure

The postprandial energy expenditure following the white bread control treatment increased acutely to peak at 30 minutes (1.076 ± 0.172 kJ/min at 30 minutes), then decreased gradually. The MS response was not different than for the white bread control; it rose quickly with a peak at 30 minutes (0.724 ± 0.314 kJ/min at 30 minutes) and then fell gradually. The WM response occurred later, with a peak at 90 minutes (0.352 ± 0.402 kJ/min at 90 minutes) before gradually decreasing. However, there were no differences in composite postprandial energy expenditure (4-hour AUC) among MS, WM, and the control.

3.5. Appetite

Hunger (Fig. 4A) and desire to eat (data not shown) decreased, and fullness (Fig. 4B) increased within 15 minutes after the consumption of each of the treatments and then progressively returned to baseline over time. These responses measured by AUC were not different among the 3 treatments (data not reported).

4. Discussion

These results indicate that the consumption of uncooked WM, a slowly digestible starch, leads to lower postprandial glucose and insulin concentrations but has no effect on postprandial energy expenditure and appetite compared to the consumption of rapidly digesting MS. These findings are similar to those of Wachters-Hagedoorn et al [8] who reported that the consumption of 50 g of available carbohydrate from a slowly digestible starch, uncooked corn starch led to smaller glucose and insulin AUC compared to 50 g of glucose.

Both uncooked normal maize starch and WM are characterized as slowly digesting starches because of their structures, which allow digestion from the inside out and a side-by-side slow digestion of the lamellar semicrystalline and amorphous layers in the granule. WM contains a greater amount of amylopectin compared with normal maize starch. The branched structure of amylopectin, which is believed to be responsible for the slow digestion effect, is densely packed in WM with perhaps somewhat less well-developed crystallites due to slightly shorter external linear chains, as well as branches that become intertwined and are more difficult to break apart. This apparently results in a steady release of glucose over an extended period [6].

The differential glucose and insulin responses between WM and MS suggest that waxy maize might be a suitable choice of carbohydrate when a slower, more prolonged release of energy is desired. In this regard, it is important to note that the participants in this study were young, lean, physically fit individuals. Our HOMA value of 16% ± 2.4% confirms that our volunteers were highly insulin sensitive. For comparison, a HOMA value of more than 47% indicates a lowered insulin sensitivity [16]. Waxy maize starch might be especially useful in food preparation for athletes. A food that resulted in a slower release of energy could eliminate the

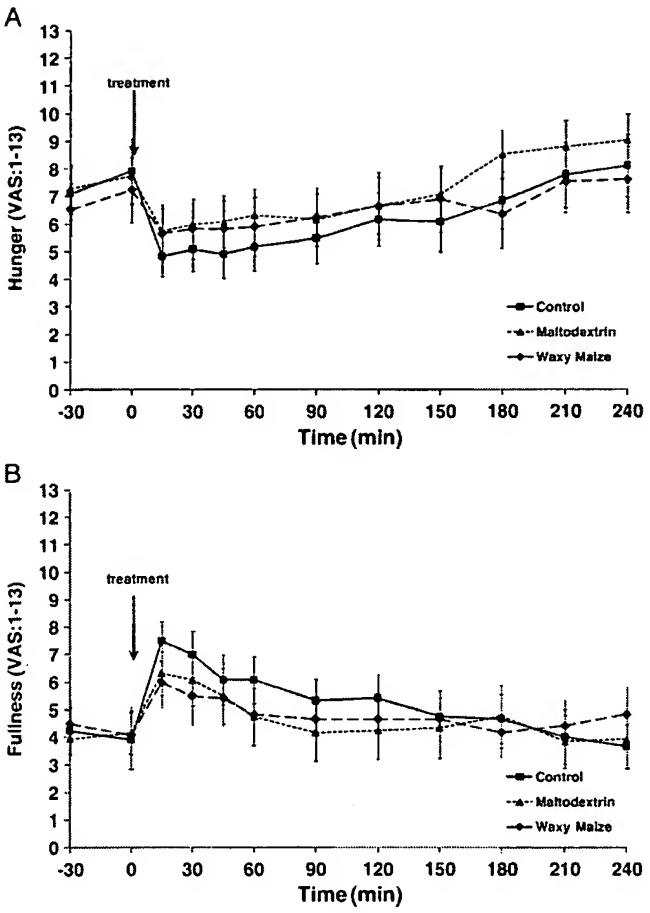

Fig. 4. Hunger and fullness responses over 4 hours after the consumption of the study treatments in 12 subjects. (A) Hunger. (B) Fullness.

potential for hypoglycemia after ingestion of a quickly digesting food. Previous research has shown that ingesting a more complex carbohydrate with a slower release of available carbohydrate results in a higher blood glucose concentration during exercise than more quickly digesting starches [2,17,18]. This higher blood glucose concentration throughout exercise can result in a longer, enhanced performance during exercise [19]. Further research is required to evaluate the effects of waxy maize on macronutrient utilization and performance during exercise. The elimination of hypoglycemia could also be useful in diabetes management, where a more controlled glucose response could help improve diabetes management and disease outcomes [7].

In this study white bread was used as a control treatment to determine a glycemic index value for the treatment containing waxy maize compared to the treatment without waxy maize. It was expected that the glycemic response of white bread would follow a course similar to that of maltodextrin; however, our results show a course more similar to the treatment containing waxy maize. This may be due to the storage conditions of the white bread. It is hypothesized that freezing and defrosting white bread may decrease the area under the curve of the glucose response [20]. It is also possible that other nutrients in the bread, besides starch, may have contributed to the lower glycemic response [21].

The decision to continue testing for 4 hours after the white bread, MS, and WM were consumed was based, in part, on the findings of Zhang et al [6]. They determined, using an in vitro model, that the glucose and insulin responses to WM should extend postprandially much longer than for fast digesting starches, and more than 2 hours were needed to document the differential responses [6]. Although the results of our study in humans indicates that the differential responses between the MS and WM primarily occurred during the first 60 min postprandial, future studies are needed to document the complete glucose and insulin response profiles. This will require continuing testing beyond 4 hours and until glucose and insulin concentrations return to baseline in all treatments.

Our findings indicated no difference in hunger or satiety among the treatments, but these results should be viewed with caution until confirmed. Granfeldt et al studied satiety responses to slowly digesting barley treatments compared to a white bread control. Barley products resulted in a higher satiety than white bread, which was found to be inversely correlated with lower glucose responses [9]. Our small sample size (n = 12) may have contributed to the inability to find a difference in hunger, desire to eat, and fullness. The observed powers for hunger, desire to eat, and fullness were found to be 0.4, 0.6, and 0.3, respectively. It is also possible that the results were affected by the food form; for example, beverages elicit a smaller satiety response than solid foods [22-24]. Each of the treatments containing starch was in a gel/semisolid form, whereas the white bread control was a solid food. We found no differences in satiety between the gel (test treatments) and solid (white bread) treatments. However, this may have been confounded by the low palatability and distaste of the treatments containing the starch as commented by the volunteers.

It has been stated that the use of 10 subjects or less may be an insufficient number to determine a reliable glycemic index in a food [25]. It is also recommended that the glycemic index be tested 2 to 3 times in each subject for a food to reduce within-subject variability [25]. Our small subject size, and only testing each treatment once in each subject, may have led to glycemic index values that are much more variable than if we had a larger sample size or repeated testing. Although our sample size (n = 12) is small, the randomized crossover design allowed us to calculate a repeated-measures ANOVA leading to more than 95% power to detect differences in our primary outcomes, 4-hour insulin and glucose, among the treatments. In spite of our small sample size, significant differences were still observed. Thus, our sample size appears to be adequate to identify differences in postmeal glucose and insulin responses.

An additional limitation pertains to the treatment compositions. The MS treatment was composed of 78% maltodextrin and 22% sucrose; thus, it is not appropriate to attribute the responses to maltodextrin alone or the differential glucose and insulin responses among treatments to the type of starch. It is highly likely that the sucrose in the MS might have affected the postprandial responses of the MS treatment. The glycemic indices of maltodextrin, sucrose, and fructose are 100, 58, and 12, respectively. The sucrose might have reduced the difference between the glycemic indices of the MS and WM treatments. Although our results are consistent with previous research in other types of slow digestible starches, they should be viewed as preliminary, and the precision and accuracy of the glycemic and insulinemic responses of waxy maize confirmed with other research.

In conclusion, these results establish in humans that the consumption of WM leads to blunted postprandial glucose and insulin responses, potentially leading to a more steady supply and release of energy over a period, compared to the rapidly digesting starch maltodextrin.

Acknowledgment

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APPENDIX G

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ORIGINAL ARTICLE

A novel starch for the treatment of glycogen storage diseases

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Summary *Objective:* To determine whether a new starch offers better short-term metabolic control than uncooked cornstarch in patients with glycogen storage diseases (GSDs). *Study design:* A short-term double-blind cross-over pilot study comparing uncooked physically modified cornstarch (WMHM20) with uncooked cornstarch in patients with GSD types Ia, Ib and III. Twenty-one patients (ages 3–47, 9 female) were given 2 g/kg cornstarch or WMHM20 mixed in water. Blood glucose, lactate and insulin, and breath hydrogen and $^{13}CO_2$ enrichment were measured, at baseline and after each load. The hourly biochemical evaluations terminated when blood glucose was ≤3.0 mmol/L, when the study period had lasted 10 h or when the patient wished to end the test. The alternative starch was administered under similar trial conditions a median of 10 days later. *Results:* The median starch load duration was 9 h for WMHM20 versus 7 h for cornstarch. Glucose decreased more slowly (p=0.05) and lactate was suppressed faster (p=0.17) for WMHM20 compared with cornstarch. Peak hydrogen excretion was increased (p=0.05) when cornstarch was taken. *Conclusion:* These data indicate longer duration of euglycaemia and better short-term metabolic control in the majority of GSD patients with WMHM20 compared to cornstarch.

Communicating editor: René Santer

Competing interests: None declared

References to electronic databases: Glycogen storage disease I, OMIM 232200. Glycogen storage disease III, OMIM 232400. Glucose-6-phosphatase, EC 3.1.3.9. Amylo-1,6-glucosidase, EC 3.2.1.33. Oligo-1,4–1,4-glucanotransferase, EC 2.4.1.25

Abbreviations

CNPF	continuous nocturnal pump feed
GSD	glycogen storage disease

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IQ	interquartile range
PDB	Pee Dee Belemnite
UCCS	uncooked cornstarch
WMHM20	Waxy Maize (Heat Modified) 20

Introduction

The glycogen storage diseases (GSDs) comprise a group of rare inherited disorders of glycogen metabolism. GSD I (OMIM 232200) is caused by reduced activity of glucose-6-phosphatase (G6Pase, EC 3.1.3.9); GSD Ia by deficiency of the hydrolytic enzyme; and GSD Ib by deficiency of the endoplasmic reticulum transmembrane glucose 6-phosphate transport protein, G6P translocase. The major metabolic consequence of ineffective function of G6Pase is hypoglycaemia, provoked by relatively short fasts. Secondary metabolic disturbances include hyperlactataemia, hyperuricaemia and hyperlipidaemia (Chen 2001). GSD III (OMIM 232400) is caused by deficiency of glycogen debrancher enzyme (EC 2.4.1.25). Many patients with GSD III are also prone to hypoglycaemic episodes after short fasts, particularly during childhood. Secondary metabolic disturbances include ketosis and hyperlipidaemia.

Maintaining blood glucose concentration in the normal range improves secondary biochemical features as well as clinical parameters. The introduction of continuous nasogastric glucose polymer feeds showed this clearly (Greene et al 1976). The subsequent introduction of uncooked cornstarch (UCCS) into the daily dietary treatment at least matched this improvement (Chen et al 1984, 1993). While the introduction of UCCS has benefited many patients, its use does have problems. For some the duration of normoglycaemia can be less than 4 h, many find the mixture neither palatable nor convenient, and for others there can be symptoms of bloating, flatulence and diarrhoea with large doses (Lee et al 1996). UCCS is only partially utilized and can be associated with malabsorption in GSD I (Bodamer et al 2002). Diarrhoea may also be a feature of GSD I itself or its treatment with cornstarch, and inflammatory bowel disease is a feature of GSD Ib (Sanderson et al 1991; Visser et al 2002).

Apart from sustained normoglycaemia without excessive insulin rise, the features of an ideal starch for treatment of patients with the hepatic GSDs include suppression of secondary biochemical abnormalities, palatability, convenience, few side-effects and maintenance of normal appetite (without excessive weight gain) (Rake et al 2002b; Smit et al 1984). We have looked at many methods of optimizing the efficacy of starch therapy, including delaying gastrointestinal transit time, enhancing digestion of starch and delaying digestion of starch. In a pilot study with one patient, (unpublished data) we found better metabolic control with a physically modified cornstarch, WMHM20 (Glycologic Ltd, Glasgow, UK; international patent WO2005044284).The physical properties of cornstarch and WMHM20 are shown in Table 1. This study was designed to assess whether this benefit was sustained in a larger group of patients. We therefore tested the hypothesis that there is longer duration of normoglycaemia with the short-term use of WMHM20 compared to cornstarch.

Table 1 Composition of starches used in study

	Cornstarch	WMHM20
Moisture content	10.9%	11.9%
Amylopectin content	72.8%	99.5%
Total carbohydrate (wet base)	84.6%	84.2%
Resistant starch (Englyst et al 1994)	60.5%	67.7%

Study design

The study was approved by the Joint Ethics Committee of The National Hospital for Neurology and Neurosurgery and Institute of Neurology, London, UK, and the Institute of Child Health/Great Ormond Street Hospital Ethics committee, London, UK. GSD I and III patients were recruited from adult and paediatric tertiary referral metabolic units in London. Written informed consent was taken from all adults above 16 years of age and from a legal guardian of children under 16 years. The diagnosis of GSD I and III was based on a liver biopsy showing reduced activity of the appropriate enzyme, a mutation in the appropriate gene or white blood cell glycogen debrancher enzyme activity indicative of GSD III. All had evidence from their medical history of fasting hypoglycaemia and were taking UCCS.

The study had a randomized double-blind crossover design. Patients anonymized by reference number were randomly allocated to receive either UCCS (National Starch & Chemical Ltd, Manchester, UK) or WMHM20. Each starch was manufactured using food-grade techniques and packaged in identical containers bearing a reference number. The patient reference numbers and container reference numbers were paired by Glycologic Ltd and the supervising physician was blinded to this pairing. Research participants were asked to re-attend for the second starch load, using the

alternative starch, a median of 10 days (IQ 7–14 days) afterwards. The supervising physician devised a safe personalized fasting period for each patient based on previous cornstarch loads and medical history. Clear instructions were given to the research subject, or their carer, for the participant to have the same diet the day before and fast interval immediately before each starch load.

Methods

Starch load test

Initially, an intravenous cannula was placed in the patient's arm and baseline blood and breath samples were collected. Then, 2 g of the nominated starch per kg body weight (maximum 120 g) was mixed in cold water and ingested. Breath and blood samples were performed hourly after the starch administration. No further intake, apart from drinking water, was allowed. The starch load test ended when the patient had fasted for 10 h, the blood glucose was ≤3.0 mmol/L on the bedside glucose monitor or the patient wished to end the test. When the blood glucose was ≤4.0 mmol/L in children aged 3–16 years, blood tests were performed at 30 min intervals until the test end.

Biochemical data

The blood samples were analysed: bedside whole-blood glucose (Advantage II, Roche diagnostics, Mannheim, Germany); laboratory plasma glucose and lactate (Vitros Fusion 5.1, Ortho-Clinical Diagnostic, High Wycombe, UK); and serum insulin was performed by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). The laboratory glucose and lactate samples were collected into lithium fluoride, transported on ice and separated within 30 min of sampling. The insulin samples were collected as a clotted sample and also separated within 30 min. The bedside glucose monitor was used as a screening tool to identify hypoglycaemia (blood glucose ≤3.0 mmol/L) and consequently determine when to end the test. Statistical analyses were performed on laboratory plasma glucose data.

Breath data

Breath hydrogen was measured immediately at the bedside using a portable hydrogen measuring device (Micro H_2, Micro Medical, Rochester, UK), while $^{13}CO_2$ breath samples were collected into a gas sampling system (Micro Medical) and the gas was transferred using a gas-tight syringe to a gas-tight 10 ml vacuum tube (Labco Ltd, High Wycombe, UK). Breath CO_2 was analysed for $^{13}CO_2/^{12}CO_2$ enrichment by gas chromatography on a CP-Poraplot-Q column (Varian Inc., Oxford, UK) followed by isotope ratio mass spectrometry on a Thermo Finnigan Delta-XP (Thermo Finnigan, Bremen, Germany). Sample $^{13}CO_2/^{12}CO_2$ enrichment was standardized against a CO_2 cylinder (5.0 grade, BOC Special Gases, Guildford, UK) calibrated against the international standard Pee Dee Belemnite (PDB) (Iso-Analytical, Sandbach, Cheshire, UK). The WMHM20 and UCCS $^{13}C/^{12}C$ ratios were analysed by elemental analyser isotope ratio mass spectrometry (Iso-Analytical). The enrichments of UCCS, WMHM20 and Maxijul glucose polymer (SHS Ltd, Liverpool, UK) after complete combustion were $\delta‰ = -11.13$, -10.75 and -11.32, respectively. UCCS and WMHM20 utilization were calculated from the $^{13}CO_2/^{12}CO_2$ ratios as described previously, substituting the above UCCS and WMHM20 enrichments in the formula (Bodamer et al 2002).

Statistics

The gradient of increase of glucose from baseline to peak and gradient of decrease of glucose from peak to the end of each starch load was assessed. Similar gradients were assessed for lactate: baseline to trough lactate and from trough to test end for each load. These paired gradients, for each starch load, were compared using a two-tailed paired *t*-test. Using nonparametric analyses, there was no statistical difference in glucose decline when comparing GSD Ia

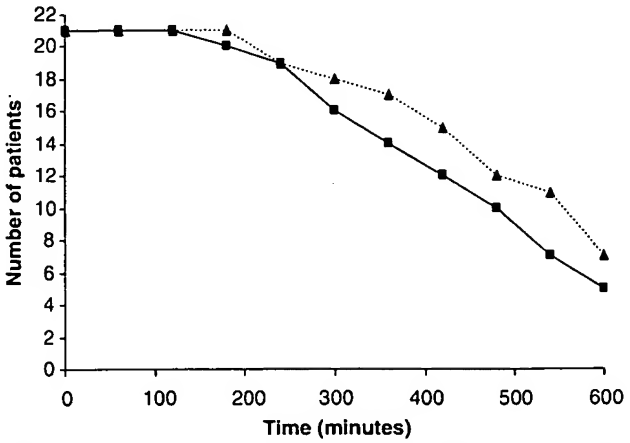
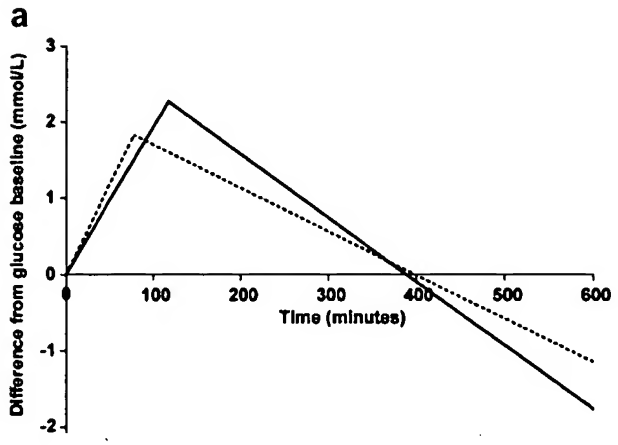


Fig. 1 Test duration for 21 patients with GSD I or III. ···▲···, WMHM20; —■—, UCCS

patients with Ib and GSD Ia with III, but there was a statistical difference between these disorders in the lactate profile. Consequently, glucose data were compared for all patients and also substratified to those with GSD I and GSD Ia only, whereas lactate data were compared for GSD I and GSD Ia only (see Fig. 2). Mean glucose oxidation breath values for each starch load were compared at 60 min intervals using a two-tailed paired *t*-test. However, it was noted using an unpaired two tailed *t*-test that there was a statistical



	Baseline to peak	*Peak to trough*
ALL	0.563	**0.0464**
GSD I	0.394	0.0820
GSD IA	0.571	0.114

	Baseline to trough	*Trough to peak*
GSD I	0.173	0.466
GSD IA	0.381	0.380

Fig. 2 Mean gradient of incline from baseline to peak and decline from peak to test end for glucose (**a**) and from baseline to trough and trough to test end lactate (**b**.)·····, WMHM20; ——, UCCS. Tables indicate *p*-values calculated from paired *t*-tests.

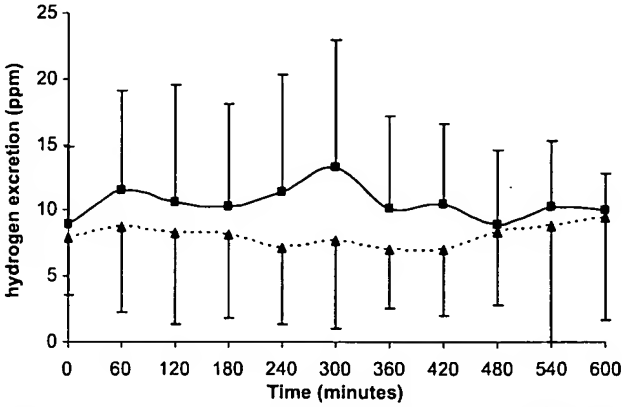


Fig. 3 Mean hydrogen excretion for patients taking WMHM20. or cornstarch (errors bars of one standard deviation). ···▲···, WMHM20; —■—, UCCS

difference (p=0.0035) in the baseline values of $^{13}CO_2$ breath enrichment of those participants who had overnight Maxijul glucose polymer pump feeds prior to the starch loads, with the mean ±1 standard deviation being (−18.4 ± 2.70‰ vs PDB) compared with those taking just UCCS (−21.6 ± 2.97.) Subsequent analysis was therefore performed on those patients who were not managed with continuous nocturnal pump feeds and fasted for 2 h or greater, before the starch load as indicated in Fig. 4. The ^{13}C content of Maxijul by complete combustion was ascertained due to this difference in baseline and is indicated above.

The mean hourly hydrogen excretion for each starch was compared during using a two tailed paired *t*-test as indicated in Fig. 3. The area under the graph for each profile was also calculated; the mean area for each cohort and comparison by paired *t*-tests is indicated in Table 2. However, the area under the curve may not be entirely representative of hydrogen excretion as shorter trials have less area than longer trials with similar excretion. Consequently, the mean hydrogen excretion per starch load is also indicated in Table 2.

Table 2 Mean area under the breath hydrogen curve and mean hydrogen excretion per patient for starch-load duration for subjects with GSD Ia and Ib able to perform breath tests (n=16) and GSD Ia (n=11.) Comparison by paired *t*-test

	Cornstarch	WMHM20	*p*-Value
Mean area under curve (ppm×min)			
GSD Ia and Ib	5220	3930	0.178
GSD Ia	4740	3940	0.434
Mean hydrogen excretion (ppm)			
GSD Ia and Ib	11.3	8.11	0.0635
GSD Ia	11.2	8.74	0.163

For the 6 children in our study who were 14 years and under who were able to perform breath tests adequately, the mean hydrogen excretion (±SD) for the duration of the studies was 3.8 ppm (±2.6) for WMHM20 and 3.5 ppm (±2.1) for UCCS. For the 12 patients who were 15 years and over, these values were 9.8 ppm (±6.1) for WHMH20 and 13.4 ppm (±6.8) for UCCS. In addition, there was statistical significance difference ($p<0.00001$) using a two-tailed unpaired t-test comparing the mean hourly excretion between the two age ranges for each starch.

Results

Patient demographics and test duration data are shown in Table 3. If the patient ended the test with a laboratory glucose ≤3.0 mmol/L, the duration of normoglycaemia is indicated by the last glucose>3.0 mmol/L, usually 1 h (occasionally 30 min) previous to the low value. Median test duration for WMHM20 was 9 h (IQ 6.0–10.0) and for UCCS was 7 h (IQ 5.0–9.0). Comparative test duration is indicated in Fig. 1. The patients ended the tests for various reasons: for WMHM20, six had genuine hypoglycaemia, two patients ended their trial because the bedside glucose monitor under-read the laboratory glucose, six terminated for personal reasons unrelated to hypoglycaemia and seven lasted the full test duration of 10 h. For UCCS, nine patients ended the test with genuine hypoglycaemia, four because the bedside glucose monitor under-read the laboratory glucose, three for personal reasons unrelated to hypoglycaemia and five patients lasted for the full test duration of 10 h. Patients as a whole had a longer period of euglycaemia using WMHM20, but 8/21 from the WMHM20 group and 7/21 from the UCCS group terminated the study prematurely. There was no significant difference in the mean area under the curve for the glucose profiles ($p=0.47$). However, the area under the curve does not necessarily represent the primary outcome of duration of normoglycaemia, as discussed later. Consequently, the gradients for each glucose and lactate profile from baseline to peak values and from peak to trough were taken as described above. There was no statistical difference for the gradient of increase in glucose, but WMHM20 had a slower glucose decline than cornstarch ($p=0.05$), in the whole cohort. There were no statistical differences in the lactate profile but the mean lactate tended to decrease faster in all GSD I patients ($p=0.17$) for WMHM20 compared with UCCS.

Table 3 Diagnosis, pre-test management, trial duration and conditions for trial termination for both WMHM20 and cornstarch

Age(years)	Sex(M/F)	GSD diagnosis	Pre-load fast (h)	Nocturnal regimen	WMHM20 (h)	T*	Cornstarch (h)	T*
3	F	Ia	<0.5	CNPF	3	h	3	h
4	F	Ia	<0.5	CNPF	4	h	4	h
5	M	Ia	<0.5	CNPF	3	h	4	h
5	M	Ia	<0.5	CNPF	7	u	4	u
7	M	Ia	<0.5	CNPF	9	p	6	u
12	M	Ia	1	CNPF	9	p	7	p*
21	M	Ia	2	CNPF	10	m	10	m
22	F	Ia	4	UCCS	10	m	9	h
22	M	Ia	1	CNPF	6	h	5	u
23	F	Ia	2	UCCS	6	h	8	h
33	M	Ia	2	UCCS	10	m	6	h
34	M	Ia	10	UCCS	10	m	10	m
47	M	Ia	12	UCCS	7	p	10	m
13	M	Ib	1	CNPF	9	p	9	p#
14	F	Ib	5.5	UCCS	10	m	8	h
15	F	Ib	3	UCCS	9	p	10	m
24	M	Ib	4	UCCS	10	m	7	u
35	M	Ib	2	UCCS	8	h+	10	m
38	F	Ib	12	UCCS	10	m	5	h+
3	F	III	<0.5	CNPF	5.5	u	2	h
12	M	III	0.5	CNPF	7	p	8	p

CNPF, continuous nocturnal pump feed; UCCS, uncooked cornstarch taken overnight.
T*: *Conditions for test end*—h=hypoglycaemia (glucose<3.0 mmol/L) confirmed on laboratory glucose; u=under-reading: bedside glucose (<3.0 mmol/L), resulting in test end, laboratory glucose>3.0 mmol/L; p=personal reasons unrelated to hypoglycaemia at test end; p*=patient had profound headache and lethargy at test end; p# =patient had profound diarrhoea at test end; h+ =patient's baseline preparation different for each test load against advice; m=maximum test duration=10 h.

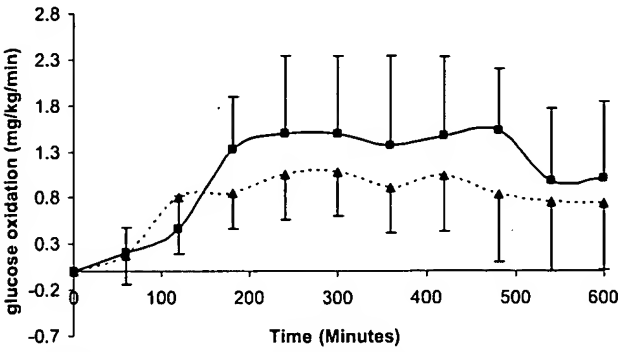


Fig. 4 Mean calculated glucose oxidation of 10 patients who had fasted for ≥2 h prior to starch load (errors bars of one standard deviation). ···▲···, WMHM20; —■—, UCCS

The mean gradients for each sector are demonstrated in Fig. 2 to illustrate how the difference applies to the glucose and lactate profiles.

There were no statistical differences noted in the insulin profile. The mean peak (±1 SD) insulin for UCCS was 15.9 IU/L (±12.3) and for WMHM20 was 13.7 IU/L (±12.2). On average, peak insulin was reached in 1 h for UCCS and in 2 h for WMHM20.

There was no statistically significant increase in utilization of UCCS compared to WMHM20 in the 10 patients who did not take overnight glucose polymer (Fig. 4). The mean hydrogen breath data are shown in Fig. 3. There was a statistically significant difference at 300 min (p=0.05), indicating greater colonic fermentation and potentially malabsorption of UCCS compared to WMHM20.

Discussion

This study compared the short-term metabolic profile of 'traditional' cornstarch and a novel physically modified cornstarch and demonstrates preliminary evidence of a more favourable outcome with the latter. We demonstrate a longer duration of action, slower decrease in glucose, more rapid suppression of lactate and less relative colonic fermentation of the novel starch compared to UCCS.

Since the identification of glucose-6-phosphatase by Cori and Cori in 1952, several strategies for ameliorating metabolic disturbances in patients with GSDs have been attempted. Portocaval shunts in the 1960s aimed to reduce hepatic first-pass metabolism of dietary glucose (Starzl et al 1965.) The use of parenteral nutrition appeared a more effective strategy (Folkman et al 1972). This was simplified further by the introduction of continuous nocturnal enteral feeds in conjunction with frequent daytime meals (Greene et al 1976; Wolfsdorf and Crigler 1999). The latter strategy demonstrated very clear improvement in growth and overall metabolic control. This treatment appears effective but it is onerous and ongoing concerns with mechanical pump failure and tube dislodgement remain (Leonard and Dunger 1978; Rake et al 2002a.) Daytime meals can be frequent (<2 h interval) and consequently several studies looked at slow glucose-releasing dietary starches to extend this period of euglycaemia (Chen et al 1984; Sidbury et al 1986; Smit et al 1988; Wolfsdorf and Crigler 1997).

The introduction of UCCS into the dietary regimen in the 1980s improved daytime management and allowed some patients to replace nocturnal pump feeds of glucose polymer with a dose of cornstarch. An important consequence of the introduction of these dietary therapies was an improved quality of life and prognosis (Moses 2002). While many patients clearly benefit from UCCS, some patients do not have sustained normoglycaemia and many have symptoms of bloating, flatulence and gastrointestinal disturbance. In some patients these symptoms may be related to incomplete digestion of starch (Bodamer et al 2002). However, it has become increasingly clear that there is substantial morbidity in older patients, including the development of hepatic adenoma and potentially hepatocellular carcinoma, renal tubular and glomerular disease and fractures related to osteopenia (Lee and Leonard 1995; Lee et al 1995a,b,c; Weinstein and Wolfsdorf 2002). While the pathophysiology of all these processes is not clear, improved primary and secondary metabolic control is associated with less morbidity. Therefore, current management protocols recommend strategies to improve metabolic control (Rake et al 2002b). For some patients, including adults, improved metabolic control continues to require frequent meals or UCCS and nocturnal nasogastric pump feeds. Such intensive strategies may have a major impact on quality of life and psychosocial well-being.

It has thus been a goal of many research groups to find a dietary starch that improves metabolic control for a sustained period. Starch is a glucose polymer. Variation in physical properties and granular organization gives a starch its unique digestibility profile. In particular, the amylose (linear chain) to amylopectin (branched chain) ratio, particle size and proportion and nature of the crystalline structure determine the digestibility of any given starch (Smit et al 1988). However, the physical properties of starch can be modified by chemical, heat, pressure or enzymatic treatment, which subsequently alters its digestibility.

The physiology of an individual also greatly determines to what extent glucose is liberated from available dietary starch. Factors such as gastrointestinal transit time, abundance of pancreatic α-amylase and gastrointestinal mucosal integrity determine the extent to which this occurs. Undigested starch undergoes fermentation by colonic bacterial flora, releasing hydrogen, which is absorbed into the bloodstream and excreted in the breath. Measurement of breath hydrogen excretion is a well-recognized technique for assessing starch malabsorption (Casellas et al 2004; Metz et al 1975).

The authors' clinical research group has approached the development of a dietary starch in a number of ways. We have tried various synthetic starches with different ratios of amylose/amylopectin but have not found the desired metabolic profile. High amylose content, for example in high-amylose maize, is difficult to digest (Tester et al 2004). The use of products with delayed gastrointestinal time and the use of pancreatic enzyme supplements have not appeared effective in our hands. However, by controlled heat–moisture processing of cornstarch, we have found that the new 'reorganized' cornstarch (WMHM20) can offer better metabolic control than traditional UCCS.

Having performed this study as a pilot study on one and then five patients from our clinic (unpublished data), we invited all patients in our clinic with GSD Ia, Ib and III who take cornstarch as part of their treatment to participate in the full study protocol to see whether there is any evidence of benefit in the broader GSD population To this end, the overall test duration of the novel starch appears beneficial for the majority of patients. The glucose and lactate profiles also appear favourable, but some of these data were not statistically significant. There was also no statistical difference in the area under the curve for each of these glucose profiles owing to the relatively higher peak glucose of the UCCS (Fig. 2a) and the shorter test durations of these patients. We believe the lower peak glucose and gradual sustained decline of the glucose curve conferred by WMHM20 is the more desirable metabolic profile, despite the equivalent area that each curve yields.

Ideally, starch load tests and hydrogen/$^{13}CO_2$ breath tests should be performed after a substantial fast to discriminate interference from other ingested substances, but this is rarely possible in patients with GSD. In addition, there should also ideally be a pre-test 'washout' of ^{13}C-containing food. This again is not possible in this study population, who are dependent on regular cornstarch with high ^{13}C content, leading to a statistically different baseline breath $^{13}CO_2$ when compared to the normal population (Bodamer et al 2002.) The best compromise was to recommend that patients' pre-test management be identical for each load, with patients acting as their own controls. We assumed that day-to-day variation was minimal on the two test days, yet this was not always the case.

There was increased hydrogen excretion compatible with increased colonic delivery and fermentation of UCCS compared to WMHM20, suggesting greater malabsorption of UCCS. If the criterion for malabsorption of peak hydrogen excretion>20 ppm was used, eight UCCS subjects and four WMHM20 met the criterion (Metz et al 1975). Increased breath hydrogen excretion was not demonstrated in two previous studies of patients with GSD I, but those studies were in children and adults all under the age of 22 years (Smit et al 1984; Visser et al 2002.) Our results indicate a lower mean hydrogen excretion for starch load duration for those patients aged 14 years and under compared with those aged 15 years and over. Hydrogen excretion seems to increase with age, implying that there is acquired malabsorption in this patient group. We cannot, however, exclude that the differences between this study and those published previously are due to differences in dose or regional variations in management. Further work is required to elucidate more precisely the physiology of starch digestion in GSDs, including fermentation and malabsorption of different starches in these patients. Our data demonstrate peak colonic fermentation of starch at 300 min. Therefore, studies to assess fermentation and utilization of starch should extend beyond this time in order to assess this variable effectively, However, our primary test endpoint was duration of normoglycaemia and consequently several subjects did not have studies that lasted beyond 300 min. These findings in our study are consequently preliminary and further analysis of each of these specific variables within separate trial protocols would be desirable.

We studied a small number of heterogeneous patients with large differences in age, burden of disease and management. In addition, different types of GSD were studied. In such a varied group of patients, who often require meticulous individualized management, it is difficult to implement a standardized trial protocol. This resulted in variation of baseline biochemistry and duration of tests, leading to difficulty with statistical analysis. The converse of stratifying data by disease, disease severity or age resulted in loss of statistical power in this cohort and inconclusive findings. For safety reasons, patients were also managed conservatively during the starch loads, resulting in premature termination of some studies. This contributed to the variations

observed, as fewer patients contribute data from their load towards the end.

The data presented indicate that WMHM20 has an improved lactate and glucose metabolic profile without concomitant increase in insulin, compared with UCCS. The data indicate greater fermentation and potentially malabsorption of UCCS. These preliminary data appear favourable for WMHM20. As such, this would be the first advance in dietary therapy for over 20 years for these disorders. It is necessary to examine the role of this novel starch as part of the standard dietary regimen of larger numbers of patients for a greater period of time. Even a modest improvement in fasting tolerance may have a clinically significant impact on quality of life for this group of patients as their feeding patterns integrate better with their peers.

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